A Textbook of

HISTOLOGY

By ALEXANDER A. MAXIMOW
Late Professor of Anatomy, University of Chicago

and WILLIAM BLOOM
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FIFTH EDITION
With 562 Illustrations, 32 in Color

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PREFACE TO THE FIFTH EDITION

The aim of this book, as in former editions, is to present the minute structure of the human body from a functional point of view. In keeping with this goal, extensive changes in text and illustrations were necessitated by advances made during the past six years by purely morphological means and through the adaptation of modern chemical and physical methods to histological problems.

This new edition has profited by the valuable comments of many teachers and the generous aid of several of my colleagues. Professor William L. Doyle has revised the section on *protoplasm* and written most of the discussion of its submicroscopic organization. Professor Clayton G. Loosli has recast the description of the respiratory portion of the lung. The chapter on bone has been rewritten in collaboration with my coworker in this field, Professor Franklin C. McLean. Professors Peter De Bruyn, Eugene M. K. Geiling, Ralph W. Gerard, Franklin C. McLean and William H. Taliaferro and Dr. Roy Grinker made many helpful suggestions for other topics.

The treatment of the hemopoietic tissues has been abbreviated and fairly extensive changes made in the sections on muscle and the endocrine glands. I have condensed parts of the chapter on the nervous tissue which was prepared for the last two editions by Professor Stephen Polyak. I have also rewritten much of the chapter on the female generative system. This chapter was revised in previous editions by Professor George W. Bartelmez, who contributed a description of early human placentation to the fourth edition.

Extensive portions of the book have been improved by the editorial skill of Mrs. Margaret Dickinson. Four of the five new colored illustrations were drawn by Mrs. E. Bohlman Patterson. Among the new black and white figures are autoradiographs, electron micrographs and photomicrographs made with the phase difference microscope. As in the earlier editions, my wife has contributed greatly to the preparation of manuscript and reading of proof.

Once more I wish to acknowledge with thanks the many valuable criticisms and suggestions I have received from histologists.

William Bloom

Chicago, Illinois
August, 1948

PREFACE TO THE FOURTH EDITION

The purpose of this book, as in the earlier editions, is to provide students with an adequate description of the minute structure of the human body and the morphologic evidences of its functions, introducing thereby as much of the data of physiology, pathology and experimental zoology as seems pertinent and desirable in the limits of the field of histology. The advances in these sciences, although hindered by the war and by the inaccessibility of much of the foreign literature, have caused such an extensive revision of this book that the text has had to be completely reset. The greatest changes are in the introduction and the chapters dealing with bone, nerve, spleen, the female generative system, and the eye. I have retained the use of large type for the general description of the topics and small type for the inclusion of further details.

The obtaining of very early implantations of chimpanzee and human ova makes it possible to include a brief description of the early stages of human placentation. Professor G. W. Bartelmez has generously contributed this new material and also a thorough revision of the rest of the chapter dealing with the female generative system.

With each revision of this book I am encouraged to face the enormous literature by the liberal help which I have received from friends and colleagues in various biological fields. I am indebted to Professor R. R. Bensley for revising the section on protoplasm, to Professor S. W. Becker for some of the changes in the chapter on the skin, to Professor E. A. Boyden for extending the description of the biliary passages, and to Professor E. M. K. Geiling for aid with the chapter on the endocrine glands. Professor S. Polyak has revised the chapters on the nervous system and the eye and has omitted some of the details of both chapters, especially of the latter. The appearance of his monograph on the retina makes it unnecessary to have the description of this structure as detailed as it was in the previous edition. Professor W. H. Taliaferro has contributed to the discussion of the macrophages and the spleen. Dr. E. Conway Mahon has helped in the revision of much of the text.

I feel particularly obligated to Professor F. C. McLean for aid in the revision of the chapter on bone and of the histophysiologic remarks in several of the other chapters.

There are thirty-five new figures, of which nine are photomicrographs, four drawings by Miss Agnes Nixon, and twenty-two drawings by Miss E. Bohlman. Among the last named are several low power, three dimensional drawings. I have added the initials A.A.M. in parentheses at the end of the legends of the figures drawn by Professor Maximow. The labels on many of the complex figures have been spelled out.

I should have liked to show the effects of more fixing and staining methods than those used for figure 2, but the differences shown will suffice for my intention at this time.

Again it is a pleasure to thank all of the histologists who have favored me with their criticisms and suggestions.

WILLIAM BLOOM

Chicago, Illinois

At the time of his death in December, 1928, Professor Maximow was writing a Text-book of Histology. This was to be based as far as possible, both as to text and figures, on human material, and the functional aspects of the structures described were to be emphasized. For this work he had collected much new material and had made many new illustrations. He had completed the sections on the male and female generative organs, the uriuary tract, the organs of special sense, and epithelium. In rough manuscript he left the sections on the blood and connective tissue, the gastro-intestinal tract, the blood vascular and lymphatic systems, the spleen, the integument, and the mammary gland.

Professor Maximow's colleagues in the Department of Anatomy felt very keenly the desirability of seeing the book completed, and at the suggestion of Professor Bensley I undertook the task.

As placentation and general embryology are treated so thoroughly in textbooks of embryology and obstetrics, Professor Maximow decided to omit descriptions of these subjects, although he included descriptions of the histogenesis of tissues and organs where they aid in understanding the structure of the mature tissue or organ. He omitted any detailed discussion of histologic technic in view of the several excellent manuals now in existence. He had originally written extensive sections on the comparative histology of each tissue and organ, but as the manuscript was becoming much larger than he had anticipated, he made drastic cuts in these sections wherever possible, and even eliminated them completely in several instances.

None of the chapters on the Nervous Tissue were written by Professor Maximow in their present form. His papers included some notes and drawings which were helpful in a general way as indicative of the line of treatment contemplated. There was also available the Russian text of 1918 in which the nervous tissues are treated very fully. A complete translation of these Russian chapters and the notes and drawings were placed in the hands of Professor Maximow's colleague, Professor C. Judson Herrick, and these served as the basis upon which the present text was written. In reorganizing this material and bringing it up to date, the attempt was made to conform with Professor Maximow's method of treatment as far as practicable; but the twelve years since the Russian text was published have been very fruitful in this field and much of the discussion which was apposite at that time is now out of date. The section dealing with the physiology of the nerve fibers and part of that on the synapse have been taken from a more complete treatment of the correlation of structure and function of nervous tissues by Professor R. W. Gerard. These chapters, accordingly, are to be regarded not as a posthumous publication, but as an entirely new formulation of the theme, the responsibility for which rests chiefly with Professor Herrick.

I am indebted to Dr. N. Hoerr for writing the description of the suprarenal bodies.

I have written the sections on the biliary and respiratory systems, the pan-

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creas, the endocrine glands (with the exception of the suprarenals) and the introductory chapter. In all of these sections I have conformed, in general, with Professor Maximow's ideas on these subjects. In addition, I have thoroughly revised the sections on cartilage, bone and muscle, which were based on translations of parts of his Principles of Histology (Russian), and his rough manuscript on the blood vascular and lymphatic systems, the spleen, integument, mammary gland, gastro-intestinal tract, the blood, connective tissue, and the blood-forming and destroying tissues.

All of the illustrations with the exception of those by Professor Maximow are indicated by initials or by acknowledgment in the accompanying legend. I have included a few "key" references: a complete bibliography would be beyond the scope of the present volume and would increase, perhaps unnecessarily, the size of the book.

Throughout the work of editing and completing this book I have profited *greatly by frequent consultations with* Professor Bensley. I wish to express to him my appreciation of the many valuable suggestions he has given me and in particular for his critical reading of the text of the introduction, pancreas, thyroid, and pituitary. I am further indebted to him for several unpublished figures for reproduction here; these are acknowledged in the appropriate legends.

I am indebted to Dr. R. McKinney for his help in copying certain of the figures and in preparation of the manuscript, and to Mr. H. A. Harris for aid in reading the proof.

Various portions of the text have benefited as a result of the critical reading by Professors P. Bailey, G. W. Bartelmez, R. W. Gerard, B. Halpert, B. C. H. Harvey, P. Kyes, P. C. Kronfeld, and C. H. Swift of the University of Chicago, and Professor B. Orban of the Chicago Dental College. I am indebted to Professors P. Bailey, G. W. Bartelmez, W. Becker, B. Halpert, W. H. Lewis, W. S. Miller, and B. Orban for their kindness in extending to me original figures for reproduction here.

While this book was in press it was suggested that several of the newer staining methods which are referred to in the text should be detailed in the book. These methods are to be found in the technicological manuals listed at the end of the first chapter—especially in that of Romeis—and in the new Handbook of Microscopical Technic edited by McClung (1929, New York). An account of the celloidin embedding and sectioning methods, which are routine in this laboratory, is to be found in the article by Maximow in the Zeit. f. wiss. Mikroskopie, 1909, volume 26, page 177.

Much of Professor Maximow's manuscript was left in Russian. For their careful translations of this and of portions of his Principles of Histology (in Russian) I am indebted to Doctors G. Hassin, O. T. Hess, and E. Piette.

I wish to express appreciation to the W. B. Saunders Company for providing splendid reproductions of illustrations.

In the course of completing the unfinished work of another man it is almost inevitable that errors and discrepancies should enter the manuscript. For these I ask the indulgence of my colleagues in the science; I shall be very grateful to have any mistakes pointed out to me.

WILLIAM BLOOM

Chicago, Illinois

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INTRODUCTION

HISTOLOGY deals with the minute structures of plant and animal bodies and the morphologic evidence of their functions.

The simple animals consist of but a single cell; in the multicellular animals, groups of more or less similar cells are combined with varying amounts of intercellular substance to form *tissues.* In the more complex animals the tissues are present as such and are also combined in various ways to form *organs. General histology* is the science of the tissues, while *special histology* or *microscopic anatomy* deals with the minute structure of the organs.

This chapter consists of a brief, general consideration of cells to afford a working basis for the descriptions of tissues and organs. No attempt will be made here to consider all of the manifold variations in the primary cellular constituents. Many of these variations are characteristic only of the lower forms, but those of the mammalian organism, and in particular of man, will receive more detailed treatment in the descriptions of the tissues and organs in which they occur.

For the analysis of the structure of the embryonic and adult animal, the cell is a convenient living unit, although it is possible that smaller units of life may exist. Relatively simple organisms, such as bacteria which lack some of the characteristics of most cell types—for their structure cannot be divided into the characteristic nucleus and cytoplasm—can carry on all of the vital functions. The invisible filtrable viruses cause diseases and are known to multiply in favorable living cells; many of them have been purified and crystallized; they have a high nucleic acid content. Stanley (1940) ends his discussion of the question of whether or not they are "alive" with the following: "With the realization that there is no definite boundary between the living and the nonliving, it becomes possible to blend the atomic theory, the germ theory, and the cell theory into a unified philosophy, the essence of which is structure or architecture. The chemical, biological, and physical properties of matter, whether atoms, molecules, germs, or cells, are directly dependent upon the chemical structure of the matter, and the results of the work with viruses have permitted the conclusion that this structure is fundamentally the same regardless of its occurrence."

Many biologists do not agree with this and regard the line between living and nonliving as more sharply drawn. In any event, the cells found in the bodies of multicellular animals show marked variations in structure and function and represent complex functional living units, which are coordinated by and subservient to the functions of the whole animal

METHODS OF STUDY

History. Although the microscope has been used for nearly three hundred years for the study of minute anatomy, modern histology began with an outburst of investigation in the third and fourth decades of the nineteenth century and received its greatest impetus from the

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and fixed cells is necessary for knowing the structure and function of particular cells and tissues.

The great mass of work done today in both normal and pathologic histology depends on the fixation of the tissues and their subsequent staining in an elective manner.[1] The different fixatives precipitate the proteins in aggregates of different sizes; many of them leave the lipids unaffected, but most of them remove the carbohydrates and a large part of the salts. Accordingly, to study all of these constituents of a cell, various *fixation methods* must be used. Some are better than others with respect to (*a*) preservation of the cells and their constituents with a minimum of visible distortion, (*b*) speed of penetration, and (*c*) subsequent application of various stains. In general, the more acid the fixative, the more the nuclear material will be clumped; as this clumping becomes very prominent after staining, there results the common belief that fixatives with picric acid or trichloracetic acid are "good" fixatives. Actually, the various fixing solutions containing neutral formalin, osmic acid, and mercuric chloride, singly or in combination, are among the best for morphological purposes. Histochemical analysis of cells usually requires special fixatives.

The next step in the preparation of fixed tissues for study consists in slicing them into very thin layers. This is usually accomplished by freezing a bit of tissue, after which it can be sectioned in a special instrument, or by infiltrating it with a solution of gelatin, paraffin, or celloidin which is later solidified so that the tissue and the embedding matrix may be sectioned together. The use of either paraffin or celloidin requires that the tissue be dehydrated in alcohol, which removes most of the lipids. The use of paraffin permits the tissues to be sectioned relatively rapidly and in very thin slices. Celloidin, on the other hand, disturbs the arrangement of the cells less and causes less shrinkage than does the paraffin method.

These thin slices may be stained to demonstrate the various parts of the cell and the intercellular substance. The most commonly used staining method—hematoxylin and eosin—stains the nucleus blue and the cyptoplasm pink. Unless otherwise noted, the figures in this book are from material fixed in Zenker-formol and stained with "H and E." **Special staining methods are necessary to demonstrate certain cellular constituents which are present in the dead cell body but are not made visible by hematoxylin and eosin.** Figure 4 shows that a single staining method does not suffice. Many staining methods have been devised; a few are indispensable but most of them are of questionable value. Although many staining reactions are primarily physical processes (see Conn), others appear to be due to chemical interactions between cellular constituents and certain dyes. The interpretations of some of these reactions are discussed by Dempsey and Wislocki.

Some of the striking differences in the effects of a few of the commonly used fixing and staining agents are shown in Fig. 4, although only three of them will be pointed out here. The ten fixed cells of this figure are from the small intestine of the same guinea pig, while cells *b* and *c* are from those of other guinea pigs. First, it is seen that the nuclei of the cells studied supravitally (*b* and *c*) consist of a sharp membrane, a prominent body called the nucleolus, and a vague network of chromatin. The cells fixed in neutral formalin (*f*, *j*) and Zenker-formol (*g*, *k*) show much the same structures, although the latter shows more chromatin material. Absolute alcohol (*d*) and the two distinctly acid fixatives (Bouin, *e*, *i*, and Zenker-acetic, *h*, *l*) show nuclei with heavily clumped chromatin which stands out prominently. Second, this figure shows

moved, the physical make-up of the cytoplasmic constituents may be investigated directly, and various reagents may be injected directly into the cell by *microdissection*. Its main drawback is that the cell frequently changes its characteristics after microsurgical intervention. Microchemical methods of importance have also been developed.

Two staining methods have been applied successfully to living animals or surviving tissues. In *vital staining*, the dyestuff is introduced into the living organism and, depending on the constitution of the dye, certain parts of the organism will accumulate this material. Examples of this procedure are the staining of the macrophages (see Fig. 9, 5, p. 12), the liver epithelium, and portions of the kidney after the intravenous injection of lithium carmine, and the filling of the bile capillaries after the intravenous injection of sodium sulfindigotate. This method, although limited in its applications, has contributed toward the solution of certain problems connected with the morphology and function of particular cells and tissues. *Supravital staining* is the addition of a dyestuff to surviving tissues. Its most prominent accomplishment is the staining of mitochondria in living cells with Janus green B and of nerve fibers and cells with methylene blue. Under certain conditions these two dyes may be vital stains.

From these examples it is apparent that progressive advances in the study of living cells and tissues depend on the discovery of methods for the continuation of the life of the cells in complex tissues after removal from the body, and of methods which enable one to distinguish various cytological constituents within the more or less optically homogeneous body of the living cell.

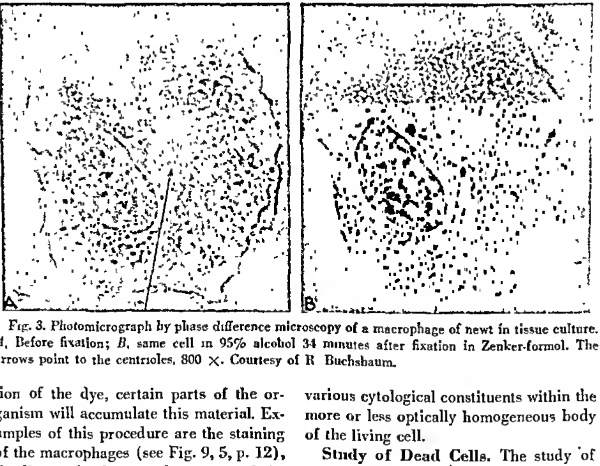

Fig. 3. Photomicrograph by phase difference microscopy of a macrophage of newt in tissue culture. *A*, Before fixation; *B*, same cell in 95% alcohol 34 minutes after fixation in Zenker-formol. The arrows point to the centrioles. 800 ×. Courtesy of R Buchsbaum.

Study of Dead Cells. The study of the living cells lacks the factor of permanency of record, except in those instances where this has been accomplished by photographic methods. This difficulty, and that of distinguishing the different parts of the cell in the living condition, have been overcome to some extent by the study of cells and tissues which have been killed, that is, "fixed," and then stained in various ways. A study of both living

the differences in the effect of these fixatives on mitochondria (see p. 10). These cellular constituents are seen with difficulty in the living cell (*c*); they are obvious as blue rods and granules after supravital staining with Janus green (*b*) and are black in the cells stained with Heidenhain's iron-hematoxylin after neutral formalin (*f*) and Zenker-formol (*g*) fixation. But they are not visible with this stain after Bouin (*e*) or Zenker-acetic (*h*) fixation. Third, mitochondria are not visible after any of these fixatives followed by staining with hematoxylin and eosin (*d, i, j, k, l*).

As the cells of the intestine change their shape greatly with the extensive movements of this organ, conclusions should not be drawn from the differences in size and shape of these cells as seen in Fig. 4 after the various fixing agents used. However, the impression that the cells and their nuclei are larger in the living than after histological preparation is correct.

By the various fixation and staining methods, many structures have been described within the cell; these are artificial to the extent that the structures in fixed material are not the same as the structures in the living cell. However, with constant fixation and staining methods, there is a constant factor of artificiality in this method of preparation. With improved methods of studying living cells, evidence has been found for the existence in the living cell of most of the important structures which had been described on the basis of fixed and stained preparations. Phase microscopy has been of great aid in this respect.

In the Altmann-Gersh freezing-drying technic, tissues are made available for chemical study almost unaltered. The movement of crystalloids and some organic substances that takes place during the application of the usual fixatives is avoided (see Figs. 10, 11). This technic has been greatly advanced by being combined with micro-incineration and with ultraviolet absorption methods.

Progress has also been made in the study of the ultra-structure of cells through the use of polarized light and x-ray diffraction spectra.

The location of radioactive isotopes in tissues can be determined by placing a photographic emulsion in contact with the section. This method, called *autoradiography*, was introduced for polonium by Lacassagne, Lattès and Lavedan. With the isolation of radioactive isotopes of more physiological interest such as those of iodine, phosphorus, carbon, and calcium, the method promises to be an important histological procedure. The precision of localization depends primarily on the range of the particles emitted, being best for the short-ranged α particles (as of plutonium, Fig. 121, A) and relatively weak β particles (I^{131}, Fig. 266; and C^{14}) and very poor for long-ranged β particles (as those of P^{32}, Fig. 121, B).

CELLULAR STRUCTURE

PROTOPLASM

Nearly all cells consist of a nucleus surrounded by cytoplasm (Fig. 5). Although the nucleus and cytoplasm have somewhat different chemical compositions, the underlying structure of both is the same "protoplasm."

The exact constitution of protoplasm is unknown. The older writers, whose work was based mainly on the appearance of protoplasm in fixed (that is, coagulated) material, held many theories as to its structure, the most important of these being the reticular, the fibrillar, the granular, and the alveolar theories. However, the apparent distribution of protoplasm in threads or granules or bubbles is due primarily to the coagulation of proteins by different agents.

Under the microscope living protoplasm presents the appearance of an optic-

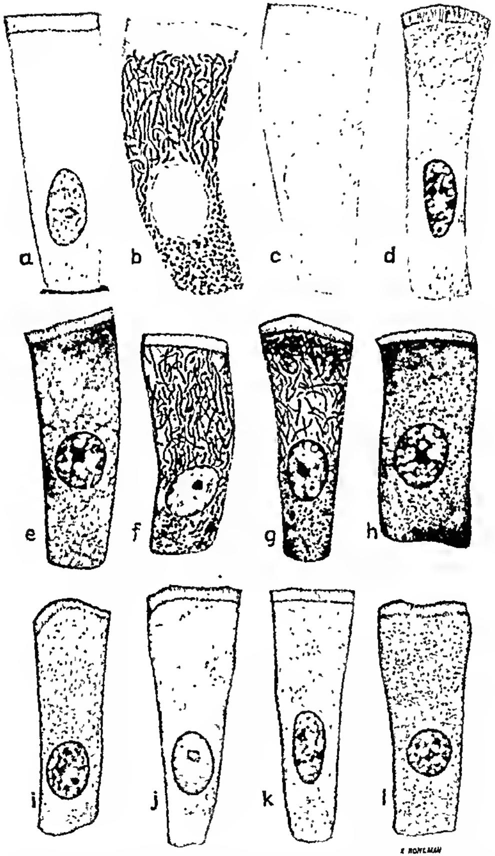

Fig. 4. Epithelial cells of small intestine of guinea pig. All fixed preparations from same animal. *a*, Zenker-formol and Mallory-azan; *b*, supravital Janus green stain for mitochondria; *c*, supravital, unstained; d, absolute alcohol and H and E; *e*, Bouin and iron hematoxylin; *f*, 10 per cent neutral formalin and iron hematoxylin; *g*, Zenker-formol and iron hematoxylin; *h*, Zenker-acetic and iron hematoxylin; *i*, Bouin and H and E; *j*, 10 per cent neutral formalin and H and E; *k*, Zenker-formol and H and E; *l*, Zenker-acetic and H and E. For explanation see text page 6. Drawn by Miss Esther Bohlman. 1620×.

deeply with basic dyes. These masses, called *chromatin granules*, seem to be suspended within the nucleus on a fine lacy framework, the *linin network*. The chromatin, in addition to the part which stains deeply with basic dyes, may be stained in part with acid dyes. Accordingly, certain authors have distinguished an *oxychromatin* and a *basichromatin*. They believe that one may turn into the other. Ultraviolet absorption studies indicate that basichromatin and also most of the oxychromatin have some content of desoxyri-

In some cases, the single nucleus may be greatly elongated and twisted in bizarre forms upon itself. This occurs characteristically in the megakarocyte of the mammalian bone marrow.

Cytoplasm. The basis of the cytoplasm is frequently spoken of as the *ground substance*. Embedded in it are the microscopically visible formed constituents of the cytoplasm: *organoids* (or *organelles*) and *inclusions*. The organoids, present in practically all cells, are probably endowed with the ability to divide,

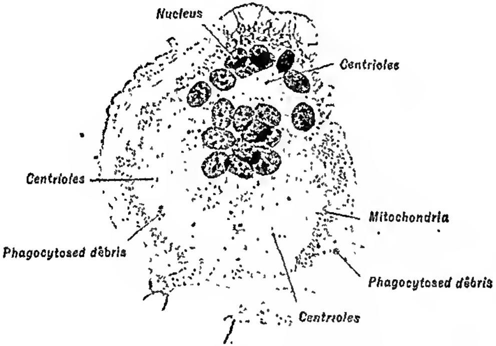

Fig. 6. Multinucleated giant cell from the newly formed connective tissue in a glass chamber placed subcutaneously in a rabbit nine days previously. Iron-hematoxylin stain. (A.A.M.)

bose nucleic acid. The nucleoli stain with acid dyes and are rich in ribose nucleic acid. They have a more or less characteristic form in particular cell types; for instance, in the hemocytoblasts they are exceedingly large. The space in the nucleus not occupied by the nucleoli, the chromatin, and the linin network, is filled with a clear liquid, the *nuclear juice*. In ultracentrifuged cells the nuclear juice is lighter than the chromatin and linin and these, in turn, than the nucleoli. Some cells contain many nuclei (Fig. 6).

and thus to perpetuate themselves; they are thus believed to be specialized particles of living substance in contrast to the inclusions which are lifeless, temporary constituents of the cell. The organoids comprise the mitochondria, the Golgi apparatus, the centrioles, and fibrils; the inclusions are accumulations of proteins, fats, carbohydrates, pigments, secretory granules, chromophil substance, and crystals. The position of the submicroscopic microsomes in this classification is uncertain (p. 14).

ally clear, continuous substance in which certain visible particles are embedded. Most of these are products of cell activity and in no sense constituents of the living substance. Some, however, such as mitochondria, may be regarded as organs of the cell.

Protoplasm is a complex liquid system surrounded by an invisible membrane which regulates interchange with the surrounding medium. The consistency of protoplasm may vary in different cells, and from moment to moment in the same cell, from that of a liquid to a rather firm gel. This change is reversible.

a cell, a new membrane is soon formed from the cytoplasm.

Between the nucleus and the cytoplasm is another membrane, called the *nuclear membrane* (Fig. 5). By microdissection it has been determined that the nuclear membrane is quite tough and slightly elastic, and that when it is punctured the nuclear content may run out, although nuclei usually "set" as a very viscous gel when injured.

The Nucleus. The nucleus of the cell is usually a globular or ovoid structure. It is slightly denser in the living cell than the surrounding protoplasm, from which

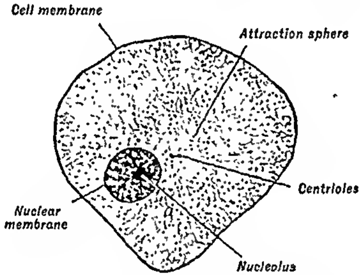

Fig. 5. Interstitial cell from the ovary of a rabbit. Iron-hematoxylin stain. 1300 ×. (A.A.M.)

Cell and Nuclear Membranes. The nature of the invisible surface membrane of the cell is still in doubt. This membrane, estimated as 100 to 200 A thick, is usually described as differentially permeable; that is, the membrane acts selectively, in unknown fashion, to permit the accumulation within the cell of some solutes and not of others. The extensive body of knowledge dealing with this topic has been thoroughly reviewed by Davson and Danielli and by Höber et al.

By microdissection it has been determined that the *cell membrane* is somewhat resistant and slightly elastic and that when it is destroyed at one point on

it is sharply delimited by the nuclear membrane. As seen in the living cell, the nucleus contains irregular wavy lines and clumps which are slightly grayer than the rest of the nucleus. Usually a distinct body, the *nucleolus* (Fig. 5), can be seen in it. The nuclear pattern of living cells is obvious in favorable phase microscope preparations (Fig. 2).

As in the case of cytoplasm, most of our knowledge of the nucleus is based on studies made on fixed and stained preparations. The nucleus is bounded by a membrane which stains deeply with basic dyes. Scattered throughout its substance are irregular masses which also stain

paratus seen in fixed preparations. This has been denied by other authors who claim to have demonstrated both the canals and the reticular apparatus in the same preparation.

When living cells are stained with a solution of neutral red, vacuoles of dye may be seen in many of them. The canalicular apparatus in mammals does not stain with neutral red.

Kirkman and Severinghaus and Hibbard give extended reviews of the literature on this organoid.

Cell Center. In most cells, usually close to the nucleus, is a condensed portion of protoplasm, called the *cell center*, or *attraction sphere*. It contains two or more small dots which can be seen in favorable living specimens with phase microscopy (Fig. 3) and which can be demonstrated readily with iron hematoxylin. They are usually close together and are called the *centrioles* or the *diplosome* (Fig. 5). The Golgi apparatus is generally located around the cell center.

The cell center and its centrioles play an important part in that form of cell division called mitosis. Occasionally, cells are found with two nuclei; these usually contain one cell center and two or more distinct diplosomes. In the relatively rare, multinucleated cells in mammals, the cell center may be quite large and may contain several isolated groups of centrioles (Fig. 6). Certain cells, as those of the nervous system, do not divide; they are seldom provided with centrioles.

Fibrils. Fine fibrils develop in many cells. They are frequently very thin and have been presumed by certain authors to offer stability to the cell. They have been called *tonofibrils* and are particularly characteristic in certain epithelial cells: in the skin they are believed to pass from one cell into its neighbors (Fig. 281). The exact nature of these structures is unknown; they probably do not develop from mitochondria. Tonofibrils disappear from a cell during mitosis and reappear in the daughter cells.

Very prominent are the fibrils found in nerve and muscle cells. The nerve fibrils have been studied for many years in tissue stained supravitally with methylene blue and in fixed and stained preparations. Microdissection studies suggest that they are present in the living nerve fiber and they have been seen in living embryonic chick ganglion cells in tissue culture. They may be displaced to one side of the cell by the ultracentrifuge.

Fibrils are one of the most characteristic features of the various types of *muscle* fibers as seen in fixed and stained preparations; although they have not been demonstrated in living cells to the satisfaction of all observers, it is quite probable that here their demonstration depends on finer methods of observation *in vivo*, as was the case with the nerve fibrils.

Inclusions.—***Proteins, Fats, and Carbohydrates.*** The inclusions of the cell may be granules of protein material, such as the dark purple-stained granules of Fig. 9, *1*, or the dark green-stained masses in *2* of the same figure. They may be of lipoid nature, as the red-stained yolk inclusions in *4* of Fig. 9. In the usual histologic preparations, however, the free fatty materials which were present in the cell are dissolved during the preparation of the section and thus appear as holes in the cells (Fig. 9, *3*). Much of the fat in the cell cannot be demonstrated microscopically, although it can be extracted chemically; this is called *masked* or *bound fat*.

Carbohydrates in the form of *glycogen* may be demonstrated in many cells, if the tissue is fixed in absolute alcohol which precipitates the glycogen. Such are the red-stained granules in the liver cells shown in Fig. 9, *3*.

Observations on living cells in tissue culture and in tissues fixed by the Altmann-

Organoids. — ***Mitochondria.*** These are small structures found in all animal cells (Fig. 7). They vary from dots to short rods, or filaments. They may be distinguished in unstained living cells, although their identification becomes much easier through the aid of Janus green B, applied supravitally (Figs. 4, 40). They are prominent in living cells studied by dark-field illumination (Fig. 8) and by phase microscopy (Fig. 2). In properly fixed preparations, they may be stained more or less electively (Figs. 9, 2 and 6). They vary from ten to several hundred in a given cell.

Fig 7. Mesenchyme cell from the subcutaneous connective tissue of four-day chick embryo, in a culture of five days. The cytoplasm contains numerous filamentous and granular mitochondria. The nucleus has a prominent nucleolus. Stained supravitally with Janus green and fixed with iodine. 1450 ×. Courtesy of W. H. Lewis.

Although mitochondria may perhaps participate indirectly in secretory processes, they probably are not transformed into secretion granules. Mitochondria, by their distribution and changes in form, undoubtedly reflect the activity of the cell. In some cases they bear a definite relationship to the polarity of the cell (Fig. 28).

Internal Reticular Apparatus of Golgi. This structure consists of a very irregularly arranged, interlacing network of fibrils, as seen in fixed preparations (Fig. 9). The network may be extensive or it may be localized in a small part of the cell. At times it may be broken up into a large number of scattered threads. In general, it is said to be of a more or less constant type in a given kind of cell and is usually localized about the cell center.

This network (often called the *Golgi network* or the *Golgi apparatus*) has been thought by many authors to play an important rôle in cellular activities, particularly those dealing with secretion. It is quite improbable, however, that the Golgi net is transformed directly into secretory vacuoles.

The Golgi net contains lipids and probably varies in composition from cell to cell. Experiments with the ultracentrifuge have shown that the Golgi apparatus is lighter and the mitochondria heavier than the rest of the cytoplasm.

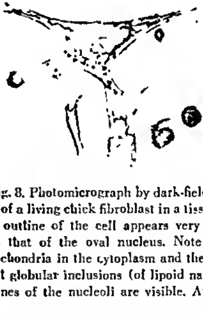

Fig. 8. Photomicrograph by dark-field illumination of a living chick fibroblast in a tissue culture. The outline of the cell appears very bright, as does that of the oval nucleus. Note the wavy mitochondria in the cytoplasm and the large and small globular inclusions (of lipoid nature). The outlines of the nucleoli are visible. After Canti.

In living cells, and occasionally in well fixed material, clear, canal-like structures can be seen. Some authors believe that the canals are identical with the Golgi ap-

Gersh freezing method suggest that glycogen is present in solution and that the granular appearance in fixed preparations is due to its precipitation by the alcohol (cf. Figs. 10 and 11). Glycogen has been separated as submicroscopic particles from suspensions of ground liver cells.

Chromophil Substance. In many cells, diffuse or discrete masses of a material which stains with the same dyes as

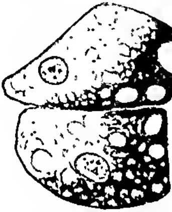

Fig. 10. Two cells from liver of amblystoma, fixed in Zenker-formol and stained with hematoxylin and Best's carmine to show glycogen. Note that this material is clumped on one side of the cell. From a preparation of I. Gersh, 400 ×. Compare with Fig. 11. (W.B.)

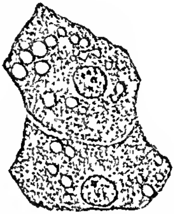

Fig. 11. Two liver cells of amblystoma, fixed by the Altmann-Gersh method and stained with hematoxylin and Best's carmine for glycogen. Note that the *glycogen is distributed evenly* throughout the cells. From a preparation of I. Gersh. 400 ×. (W. B.)

does the nuclear chromatin may be found in the cytoplasm. This material was accordingly called chromophil substance; the most prominent examples are the Nissl substance in nerve cells and the chromophil substance in the salivary glands. This material, which is apparently ribose nucleoprotein, changes greatly during the activity of the cell. The basophilia of the cytoplasm of certain rapidly dividing cells is due to a high content of ribose nucleic acid components and is probably associated with protein synthesis.

Pigment Granules. Many cells scattered throughout the body in characteristic positions, particularly in the Amphibia, contain large amounts of pigment granules, usually a melanin (Fig. 9, *7*). These cells are usually called *chromatophores*, or *melanophores*, depending upon their origin.

Crystals. Peculiar crystals, probably arising from proteins, are found in several types of cells (see Fig. 458). Their function is unknown.

Secretory Granules. In many epithelial cells various secretory granules occur. These differ cyclically in the same type of cell, depending on the stage of

5, Macrophage from the subcutaneous connective tissue of a white rat, stained intravitally with isamine blue. 1200 ×.

6, A fibroblast with rod-shaped mitochondria from the subcutaneous connective tissue of a white rat. 1200 ×.

7, Chromatophore from an axolotl embryo, with pigment granules and pink-stained yolk granules. Eosin-azure stain. 600 ×.

8, First stage in the elaboration of secretion granules (red) in the pelvic gland of Triton taeniatus. The granules first appear near the Golgi net (stained gray). The few granules near the free border of the lowest cell are from the preceding secretory cycle. Fixation Champy, stained with the Altmann method and aurantia. (Figures *5* and *6* are from preparations of Tschaschin and *8* is redrawn after Nassonow.) (A.A.M.)

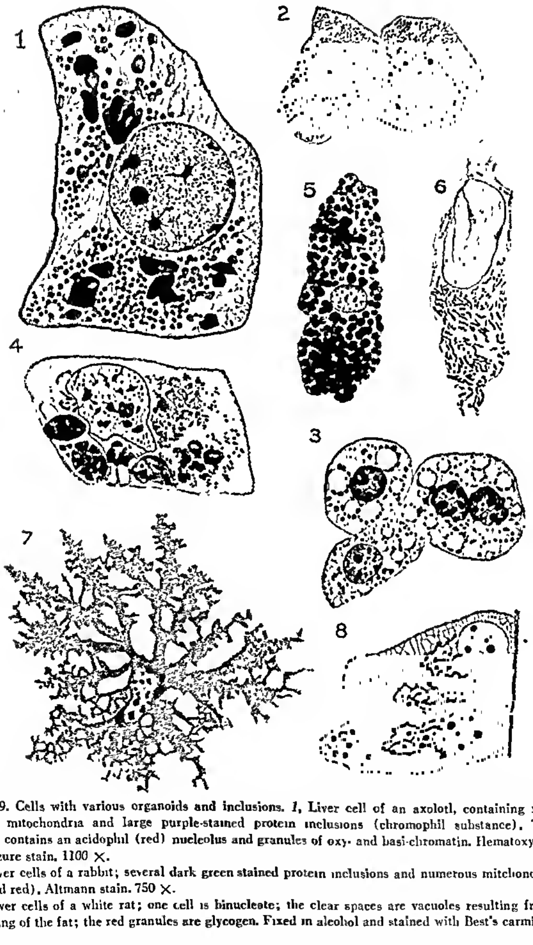

Fig. 9. Cells with various organoids and inclusions. *1*, Liver cell of an axolotl, containing red-stained mitochondria and large purple-stained protein inclusions (chromophil substance). The nucleus contains an acidophil (red) nucleolus and granules of oxy- and basi-chromatin. Hematoxylin-eosin-azure stain. 1100 ×.

2, Liver cells of a rabbit; several dark green stained protein inclusions and numerous mitchondria (stained red). Altmann stain. 750 ×.

3, Liver cells of a white rat; one cell is binucleate; the clear spaces are vacuoles resulting from dissolving of the fat; the red granules are glycogen. Fixed in alcohol and stained with Best's carmine. 800 ×.

4, Epithelial cell, from the oral cavity of an axolotl embryo, containing dark pigment granules and red-stained yolk inclusions. Eosin-azure stain. 1200 ×.

analyze cellular structure and function in the terms of these sciences. Indeed, as Schmitt states in his discussion on "Ultrastructure and the Problem of Cellular Organization": "The task therefore resolves itself largely into one of molecular and macromolecular morphology. At this level of organization the boundaries between morphology, physiology and biochemistry largely disappear."

As the cell may be considered to be an organized set of systems in dynamic equilibrium with their environments, it is not surprising that many of the common elements are to be found in protoplasm. The human body has the following percentage composition on a fresh weight basis: oxygen 65, carbon 18, hydrogen 10, nitrogen 3, calcium 2, phosphorus 1.1, potassium .35, sulfur .25, sodium .15, chlorine .15, magnesium .05 iron .004. In addition to these, traces of two dozen or so other elements normally found in living organisms are vital to life. However, on the basis of the number of molecules and ions present, a table of the composition of the body would have a different aspect; thus there are 1.7 times more hydrogen atoms in the body than all the other atoms put together.

An analysis of protoplasm reveals the presence of water, inorganic ions and innumerable naturally occurring organic compounds, some of which may be broadly classed as proteins, carbohydrates, lipid substances, their combinations, their constituents and their precursors. Preeminent in the architecture of cells are the proteins.

There are proteins which vary from cell type to cell type and are specific for organ and species. There are other proteins which seem to be common to all the cells of an individual. Important constituents of nucleus and cytoplasm are the *nucleoproteins*. Carbohydrates occur in animal cells as *glycogen* and its hydrolytic products and also combined with proteins and lipids. Intracellular fats vary from minute droplets of neutral fats in many types of cells to large accumulations in the fat cells which are specialized for the storage of these substances. Although more complex lipids as sterols and phosphatids are widely dispersed in cells, only rarely can they be demonstrated by visual means.

Protoplasm contains much potassium, very little sodium, small amounts of magnesium, and even less calcium; the heavy metals are present in traces. Of the anions, bicarbonate and phosphate predominate; chloride is present in very small amounts if at all. This contrasts strongly with the body fluids in which sodium salts, especially the chloride, predominate (Fig. 36).

It is frequently stated that protoplasm is a complex colloidal system which provides a variety of interfaces and phase boundaries at which biochemical reactions take place. But such a concept is hardly adequate in view of the degree of integration of the activities which exists in living systems. It has therefore been postulated that cells have some kind of macromolecular skeleton which provides numerous surfaces of highly specific configuration arranged as a more or less continuous phase permeated by components in true solution. If such a "cytoskeleton" exists it would have to be in dynamic equilibrium with the more fluid continuous phase. In a sense the architecture of the cell is that of a factory carrying out certain processes in connection with certain specialized structures, but it is a factory in which the raw materials, the machinery, and the end products are continually interchanging.

The use of isotopic tracers has led to the view that cellular structures and their chemical reaction systems are in dynamic equilibrium with an intracellular pool of metabolites composed of many small organic molecules. Thus the significance of

secretory activity of the cell. In some cases, the nature of these granules has been correlated with the chemical composition of the secretion, but in most cases their exact nature is not known. The secretory granules in certain cells contain antecedents of enzymes (*pro-enzymes* or *zymogen granules*). After they have left the cell, they become active enzymes. These small granules gradually progress toward the free border of the cell where, it is claimed, they imbibe water and become vacuoles, after which they are discharged from the cell.

The origin of the secretory granules is unknown; it is unlikely that they arise through a metamorphosis either of the mitochondria or of the Golgi apparatus; nor has it been shown that they develop from nuclear material or from the chromophil substance.

Submicroscopic Particles (Microsomes). By differential centrifugation and by electron micrographic study, submicroscopic particles have been isolated as cellular constituents. They vary in size from 50 to 250 Å and undoubtedly comprise a variety of materials. In the liver, some of them are glycogen, others probably are proteins. Until more is known of their chemical constitution it will not be possible to classify them more accurately; at present it would seem that some of them fall into the category of inclusions.

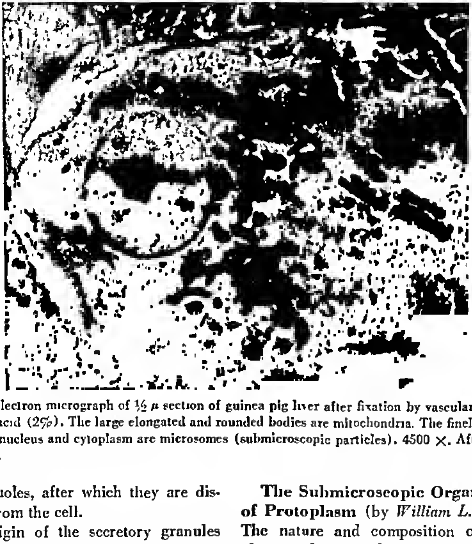

Fig. 12. Electron micrograph of ½ μ section of guinea pig liver after fixation by vascular perfusion with osmic acid (2%). The large elongated and rounded bodies are mitochondria. The finely granular material in nucleus and cytoplasm are microsomes (submicroscopic particles). 4500 ×. After Claude and Fullam.

The Submicroscopic Organization of Protoplasm (by *William L. Doyle*). The nature and composition of protoplasm and its modifications are, in the final analysis, the objects of numerous biological investigations; for, in essence, protoplasm means life. The chemical and physical analyses of cells and extracellular substance constitute the subject matter of biochemistry and biophysics. It is one of the functions of the histologist to

analyze cellular structure and function in the terms of these sciences. Indeed, as Schmitt states in his discussion on "Ultrastructure and the Problem of Cellular Organization": "The task therefore resolves itself largely into one of molecular and macromolecular morphology. At this level of organization the boundaries between morphology, physiology and biochemistry largely disappear."

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the classical distinction between endogenous metabolism and exogenous metabolism disappears (Schoenheimer).

In the transformation from food substance to constituents of living protoplasm and in the numerous metabolic processes furnishing energy, a fundamental component of each of the mechanisms is the *enzyme* which catalyzes the reaction. Now the variety of systems which operate simultaneously in a single cell is relatively large compared to the number of distinct morphological structures which we can distinguish optically. Furthermore, since in many metabolic reactions there must be a coupling between the energy producing (*exergonic*) oxidative reactions and the energy using (*endergonic*) synthetic processes, some sort of structural organization, such as a "cytoskeleton," is required to compartmentalize the processes. This concept of a cytoarchitecture is compatible with the observed specificity of many enzymes. Among the more important groups of enzymes are those catalyzing *oxidations, reductions, hydrolyses* and *phosphorylations;* esters are split by *esterases* and peptide bonds by *peptidases,* etc. It may be assumed that reversal of the hydrolytic processes is frequently accompanied by (coupled to) exergonic reactions.

Several qualitative histochemical methods have demonstrated the presence of particular enzymes, notably *phosphatase,* within cells and tissues. It is, however, difficult to deduce the physiological role of the enzymes from their localization in relatively high concentration (cf. Danielli). Actually, the demonstration of a spatial correlation between morphological units and chemical mechanisms involves numerous difficulties of practical and theoretical nature. A . ge literature of circumstantial evidence relating the configuration of cellular structures to processes significant in particular tissues is accumulating rapidly. (See reviews by Dempsey and Wislocki; Moog.) It is also evident from the biochemical differences observed between the metabolism of tissue slices, minces, and extracts that certain processes are inactivated (and others initiated) when cellular organization is disrupted. Only after suitable precautions may the structural elements be isolated in more or less intact condition for examination of their chemical mechanisms.

In multicellular animals, and more particularly in the higher forms, there is a wide variety of cells specialized for the execution of particular functions. These may be classified as dealing primarily with (1) the *vegetative existence* of the cell, (2) its *growth and reproduction,* and (3) its *special functions.* The vegetative activities of the cell, defined as the minimum of activities necessary for its continued existence, are concerned chiefly with its energy metabolism (or respiration), with the assimilation of food and the elimination of waste materials.

The processes by which energy is released from foodstuffs and made available for the needs of the individual cell and of the organism as a whole involve the consumption of oxygen, the combustion of organic molecules, and the production of carbon dioxide and water. Oxidation occurs either as the result of the addition of oxygen to a compound or of the loss of hydrogen from that compound. Cellular oxidation occurs largely as a result of dehydrogenation. In the presence of a *dehydrogenase* a molecule of foodstuff is activated in such a manner that hydrogen is transferred to a reversible oxidation-reduction system (*co-enzyme*) which becomes reduced. Hydrogen is transported from the reduced co-enzymes to other enzymes which contain iron and, in the presence of these, it combines with oxygen. Carbon dioxide production probably occurs as the result of spontaneous or enzymatic decarboxylation of certain metabolic intermediates. The co-enzymes are

thermostable, nonprotein, organic molecules which are derived from the water soluble vitamins. In general, it is likely that vitamins and minerals which are ingested in traces function as co-enzymes for various cellular enzymes.

By differential centrifugation of suspensions of cell fragments, one may obtain fractions which consist almost entirely of nuclei or mitochondria or microsomes (submicroscopic granules) and certain other granules. By this means mitochondria of guinea pig ,liver have been isolated and found to contain 65 per cent protein by dry weight and 35 per cent of fatty substances, high in phospholipids. Analyses of mitochondria of liver of other animals have given similar results. Mitochondria probably vary in composition according to the cell type in which they are found.

The relation between mitochondria and enzymes has been extensively investigated but it is only in a few instances that definite quantitative evidence has been presented. Most, if not all, of the cytochrome oxidase and succinic acid oxidase activity has been found to be associated with mitochondria. On the other hand, in many cells the enyzmes hydrolyzing dipeptides are uniformly distributed in the cytoplasm rather than localized on visible granules. The same is true for catalase which decomposes hydrogen peroxide. However, the enzyme amylase in ameba was found not to be uniformly distributed in the cytoplasm.

Numerous syntheses take place within cells. To a certain degree, as in the elaboration of proteins for the protoplasm of the individual cell, these syntheses are common to all cells, but there is also a high degree of specialization in the synthetic processes carried on within cells. The degree to which the organism as a whole can provide for its complex needs is indicated by the facts that animals can be kept alive and in excellent health with all dietary nitrogen furnished in the form of pure amino acids, and that many of the amino acids themselves can be synthesized in the body. Similarly, the animal organism can make transformations between proteins, fats and carbohydrates; indeed, it can synthesize carbohydrate from carbon dioxide and water.

The end products of metabolism are chiefly carbon dioxide, water and urea. These substances diffuse readily through cell membranes, and no process, other than diffusion, is necessary for the individual cell to rid itself of these end products. In the higher animals, however, many cells are specialized for the removal of waste products from the body.

Nucleic Acids. Nucleic acids are of primary significance in the cell; they fall into two major categories: (1) *Thymonucleic* acid (*desoxyribonucleic* acid) is composed of 4 nucleotides, each of which contains phosphoric acid, desoxyribose and one of the 4 bases thymine, adenine, cytosine and guanidine; (2) *ribonucleic* acid (yeast nucleic acid) is similarly constituted with ribose as the sugar and with uracil replacing thymine. Nucleic acids occur naturally combined with basic proteins in the form of nucleoprotein. Dilute solutions of nucleoproteins are very viscous.

Ultraviolet microscopic absorption spectroscopy as developed by Caspersson, has provided a useful device for analysis of the distribution of nucleic acids in the nucleus and cytoplasm. Studies by this method, coupled with analytical fractionation procedures and the use of specific enzymes (*ribonuclease* by Brachet) have gone hand in hand with advances in microbiology and genetics to indicate the chemical constitution and biochemical role of nucleoproteins in the nucleus and in the cytoplasm.

The demonstration of variations in the concentration of nucleoproteins (actually of purine and pyrimidine bases) in nu-

cleus and cytoplasm has resulted in theories involving nucleoproteins in the synthesis of proteins (see especially Hydén). But since we are still in ignorance of the manner of formation of a simple peptide bond, any theories on the mechanism of protein synthesis must be considered as tentative. *Plasmosin*, *renosin*, and *hepatosin* were found to be organ constituents after isolation by the methods used by Bensley and Szent-Györgyi. Further refinements of the procedures led Pollister and Mirsky to extract from the

Fig. 13. Dark-field photomicrograph of plasmosin fibers. 22 ×. After Bensley (1938).

organs a substance they called *chromosin*, which they consider to be a constituent of chromatin and to contain polymerized desoxyribonucleic acid. The term genoprotein T has been given by Stern to the desoxyribonucleoprotein obtained from thymus by a method claimed to yield the substance in the native state.

Experiments involving the quantitative analysis of nucleoproteins of tissues, notably of liver, have shown that these substances in the cytoplasm and in the nucleus exchange radioactive phosphorus with the inorganic phosphate of the cell. According to some authors ribonucleic acid is the normal precursor of desoxyribonucleic acid, but this has been denied by others. (See Brachet for nucleoproteins during development.)

CELL DIVISION

The usual mode of cell division is by a complex process called *mitosis* or *indirect division* in contrast to the much less frequent *direct division* or *amitosis*.

Mitosis. The most prominent parts in this process are played, at least at first, by the centrioles and the nucleus. Mitosis consists of four main stages: 1. *Prophase*. The centrioles separate from each other with the formation of attraction spheres around each of them while the chromatin masses change into *chromosomes* whose number is constant in a given species, and is 48 in man (Painter). 2. *Metaphase*. Each chromosome splits into two equal parts. 3. *Anaphase*. The halves of a particular chromosome separate and each half approaches one of the two centrioles which have migrated in the meantime to opposite poles of the cell. 4. *Telophase*. The formation of the two daughter nuclei and the division of the cytoplasm into masses surrounding each daugther nucleus. Wassermann (1929) recognizes five main stages in mitosis: (1) *prophase*, (2) *metakinesis*, or movement of chromosomes into (3) *metaphase*, (4) *diakinesis*, or movement of the chromosomes into (5) *telophase*. He thus separates two kinetic periods from the three relatively static stages.

The details of mitosis can best be understood by a study of the accompanying diagram (Fig. 14). In this schema, *a* indicates a *resting cell;* it shows the nucleus with its oxy- and basichromatin, a round nucleolus, and the attraction sphere with two connected centrioles. In the *prophase* (*b*) the centrioles separate, the formation of the attraction rays or asters takes place about them, and a great change develops in the nucleus by the chromatin becoming arranged into long threads; this is called the *spireme stage* of the nucleus (Fig. 15).

On comparing *a* and *b*, it becomes obvious that there has been a great increase in basophil material. This is explained as being due to the conversion of oxychromatin into basichromatin. But ultraviolet absorption studies which do not distinguish oxy- and basichromatin, show that there is an increase of nucleic acid in the nucleus during the prophase, at least during spermatogenesis.

During the transition from the prophase to the *metaphase* (*c*) the nuclear membrane dissolves. At this stage the rays emanating from the region of the centrioles are beginning to penetrate the area occupied by the chromatin threads.

In the metaphase (*d*) the astral lines radiating from the two attraction spheres about the centrioles extend in all directions and are particularly numerous in the zone connecting the two centrioles. This zone is known as the *spindle*. Suspended in the spindle are the chromosomes (chromatin threads) which at this time lie in one plane called the *equatorial plate*, midway between the centrioles. Each of the chromosomes splits longitudinally; this is perhaps the most striking part of the whole phenomenon. Although the mechanism of this longitudinal splitting is unknown, it is of fundamental importance with regard to some of our ideas on the mechanism of heredity.

In the next stage, the *anaphase* (e, *f*), the halves of each of the chromosomes separate and are drawn, or at least moved away, from each other toward the two centrioles and, at the same time, the single centrioles in each half of the cell divide into two; this is in preparation for the next cell division which, however, may be some time off. It may be recalled that in the resting cell there are usually at least two centrioles.

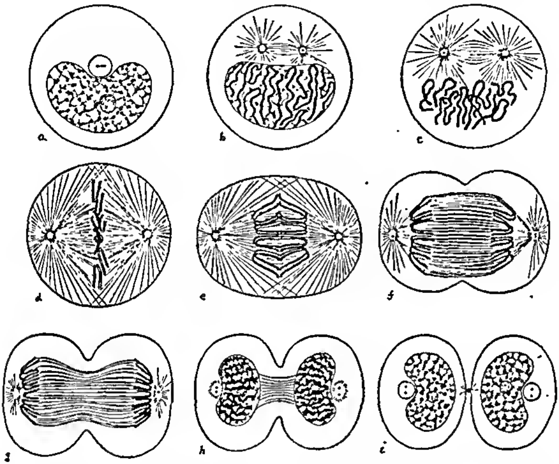

Fig. 14. Diagram showing the various stages of mitosis: *a*, Resting cell; *b*, prophase (spireme); *c*, transition from prophase to metaphase; *d*, metaphase, longitudinal splitting of chromosomes; *e*, *f*, *g*, anaphase; e, beginning separation of the split chromosomes; *f*, diaster; *g*, chromosomes approaching the centrioles; *h*, telophase with reconstruction of the daughter nuclei; *i*, daughter cells connected only by the intermediate body of Flemming.

In the last stage, that of the *telophase*, the two daughter nuclei begin to form. As the chromatin threads or chromosomes begin to break up into irregular bodies, nucleoli appear in each of the daughter cells and, through a progressive constriction of the cytoplasm (*i*), the two daughter

cells separate from each other, being attached for a time by a small acidophil body which stains black with iron hematoxylin. This is the *intermediate body* of Flemming.

The above description of the various phases of indirect division was obtained from the study of fixed and stained material. With the aid of the tissue culture method it has been shown that this process can actually be observed in the living cells. Figure 18 represents repeated drawings

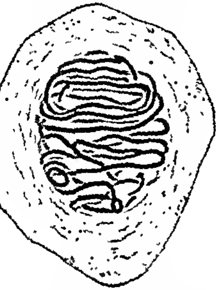

Fig. 15. Spireme of a spermatogonium of a salamander. 1000 ×. Redrawn after Prenant.

made at frequent intervals of the same cell in a tissue culture.

Of particular interest in this process, as seen in living cells, is the appearance at the cell periphery of short cytoplasmic processes. These are extruded and then retracted from various parts of the cell. These changes in the dividing cell, when studied by means of moving pictures projected much faster than they were photographed, are so violent that they have been described as "bubbling."

The cleavage of cytoplasm in animal cells usually occurs by a constriction; the various inclusions and organoids of the cell body are distributed to the two daughter cells in more or less equal amounts.

In *multipolar mitosis* there are more poles than the two which are normally present. With few exceptions multipolar mitosis is an indication of a pathologic process.

In the living, resting nucleus, the chromatin masses seem very indistinct except with phase microscopy. Direct observation of *dividing nuclei* by ordinary microscopy shows the chromatin masses very easily (Fig. 18).

As a result of mitosis, the chromatin material, which is generally regarded as the carrier of the hereditary factors of the cell and of the organism, is divided equally between the two daughter nuclei; this depends primarily upon the longi-

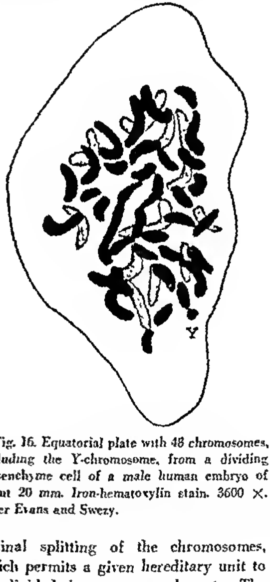

Fig. 16. Equatorial plate with 48 chromosomes, including the Y-chromosome, from a dividing mesenchyme cell of a male human embryo of about 20 mm. Iron-hematoxylin stain. 3600 ×. After Evans and Swezy.

tudinal splitting of the chromosomes, which permits a given hereditary unit to be divided into two equal parts. The mechanism causing this division of the chromosomes—as indeed of the whole mitotic process—is unknown.

For details of the participation of the chromosomes in the mechanism of heredity, the reader is referred to general works on cytology and heredity and espe-

cially to the book of Sinnott and Dunn. The mapping of the genes in the giant chromosomes of the salivary glands of Drosophila is described in the papers of Painter and of Bridges. Ultraviolet absorption studies have shown that the dark bands in these chromosomes are rich in nucleic acid and the pale ones free of it. The bands may be clearly seen in some living cells with phase microscopy (Fig. 19). The peculiar divisions which occur during the development of the sex cells are discussed in the section on the Genital Organs.

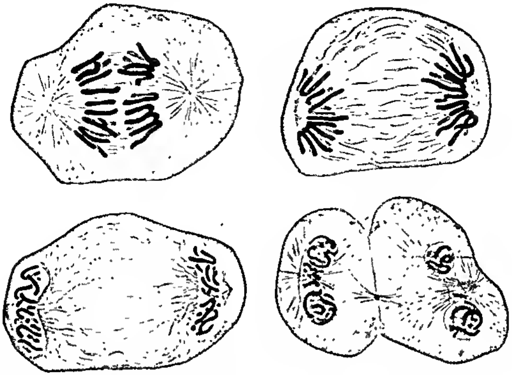

Fig. 17. Dividing spermatogonia of a salamander. Above, two stages in the anaphase; below, two stages in the telophase. 1000 ×. Redrawn after Prenant.

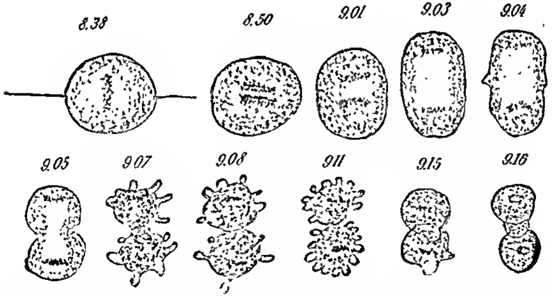

Fig. 18. Successive stages in the mitosis of a mesenchymal cell from a chick embryo of seven days, in tissue culture. The figures indicate the time at which the drawings were made from the living cell. *After Levi.*

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tion, the epithelial tissue produces special structures called glands. (See Chapter XIII.)

Early histologists described epithelium as a layer of closely connected cells lining the cavities of the body. The term was later extended to the similar layers covering the body, and was further broadened when it was shown that the vertebrate (and most invertebrate) embryos pass through a stage during which they consist of three simple layers of cells—the embryonic germ layers called *ectoderm*, *mesoderm* and *entoderm*. As the embryo develops, these epithelial germ layers are profoundly modified to form the organs and tissues of the body. In some parts they persist as epithelial layers; in other parts they lose their epithelial arrangement permanently, while in still others the epithelial arrangement seems to be completely lost, but may become apparent in inflammation, tissue culture, or tumor. If the term epithelium is used in a purely descriptive sense, as above, the student will often find it difficult to understand how the cells of many glands can be called epithelium. Accordingly, it might be advisable in many cases to distinguish between covering epithelium and glandular epithelium.

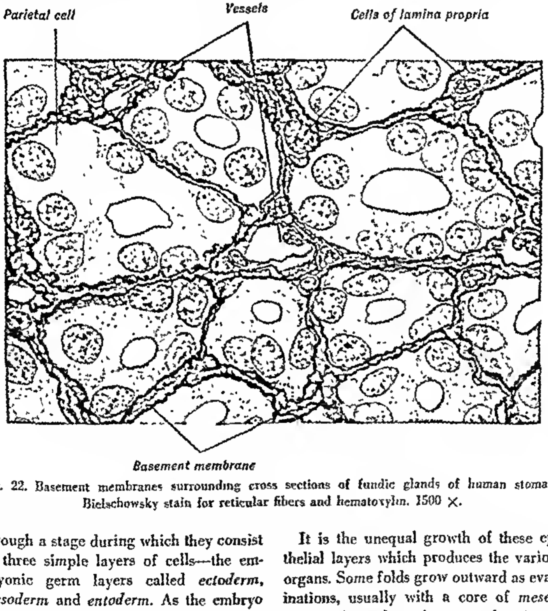

Fig. 22. Basement membranes surrounding cross sections of fundic glands of human stomach. Bielschowsky stain for reticular fibers and hematoxylin. 1500 ×.

It is the unequal growth of these epithelial layers which produces the various organs. Some folds grow outward as evaginations, usually with a core of *mesenchyme*, the embryonic connective tissue; others grow inward as invaginations into the mesenchyme. An invagination may fuse at its mouth, severing its connection with the epithelium from which it developed, and so give rise to isolated epithelial organs surrounded by mesenchyme.

EPITHELIUM

Epithelium usually consists of closely connected cells with but small amounts of intercellular substance. It forms the outer protecting surface of the body and all the glands, furnishes important parts of the sense organs, and lines the walls of the internal cavities, except those which develop exclusively in and from the mesenchyme, where the lining epithelium is called *endothelium* or *mesothelium* (p. 38). The most important and general function of the epithelial tissue is its participation in the metabolism of the body through the absorption of substances from the outside medium, their modification in the body, and the elimination of other substances to the outside. All substances normally received and given off by the body must pass through the epithelium. For the performance of the secretory func-

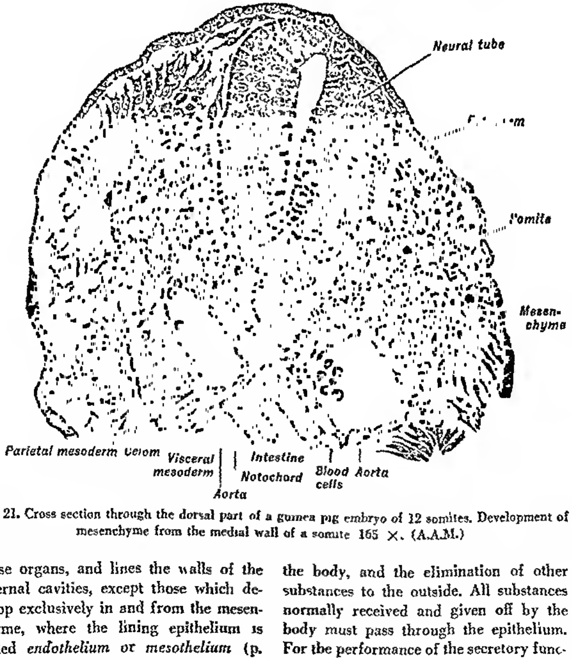

Fig. 21. Cross section through the dorsal part of a guinea pig embryo of 12 somites. Development of mesenchyme from the medial wall of a somite 165 ×. (A.A.M.)

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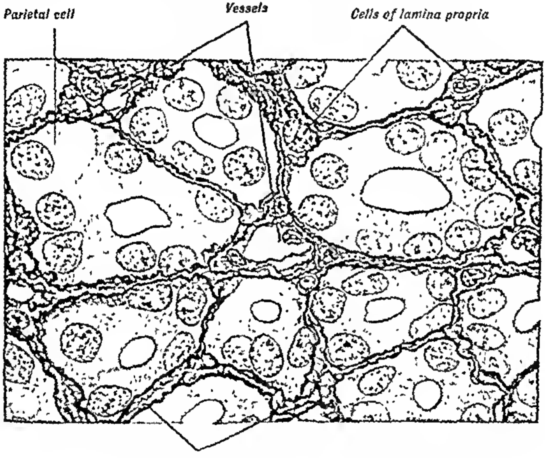

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Part of the outer germ layer, or *ectoderm*, curves inward to form the *neural tube* from which develop the elements of the brain and spinal cord. The rest of the ectoderm keeps its epithelial nature and gives rise to the epidermis, the lining of the oral cavity, and parts of the sense organs.

The inner layer, the *entoderm*, furnishes the epithelial lining and the glands of the intestinal and respiratory systems.

The middle layer or *mesoderm* keeps its epithelial character in the epithelium of

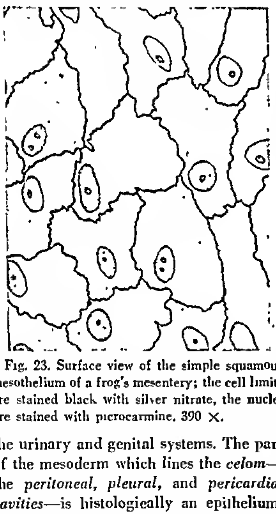

Fig. 23. Surface view of the simple squamous mesothelium of a frog's mesentery; the cell limits are stained black with silver nitrate, the nuclei are stained with picrocarmine. 390 ×.

the urinary and genital systems. The part of the mesoderm which lines the *celom*—the *peritoneal, pleural,* and *pericardial cavities*—is histologically an epithelium, but differs in some respects from the other types of this tissue and is called *mesothelium*. A considerable part of the mesoderm is transformed into the striated muscular tissue and into the heart muscle, while another large part of it becomes the mesenchyme which gives rise to smooth muscle and the various types of connective tissue, cartilage, bone and blood.

The *notochord*, the axial supporting structure of the embryo, probably arises from the totipotent tissue of the blastophore and behaves like a mesodermal derivative.

Experimental studies of vertebrate development, especially as carried out on amphibians and to a lesser extent on birds, show that many of the cells in these embryonic germ layers have no inherent specificity during early developmental stages. That is if they are moved from one place to another they will often proceed to develop in conformity with their new surroundings. These and similar interesting problems of development are discussed at length in the books by Weiss and Child, referred to at the end of the previous chapter.

Basement Membrane. Between the epithelium and the underlying connective tissue there is usually a distinct basement membrane. This is a condensation of the intercellular substance of the connective tissue at the surface of its contact with the epithelium and consists of an amorphous ground substance and reticular fibers (p. 30). In hematoxylin and eosin preparations the basement membrane often is hard to see, but special technics demonstrate it readily. In a cross section it appears as a definite boundary line of variable thickness between the two tissues (Figs. 22, 26).

Some types of epithelium seem to lack a basement membrane (thyroid gland, excretory passages of the urinary system). In the sulcus spiralis externus and in the stria vascularis of the cochlea, the epithelial cells send processes deep into the connective tissue. The original, simple, anatomical relations between epithelium and connective tissue are also completely altered in most of the endocrine glands and in the liver.

On the epithelial surface, the basement membrane at some places (skin, cornea) is provided with minute indentations into which fit corresponding, short outgrowths of the basal surface of the epithelial cells. In other places, as in the convoluted uriniferous tubules, the inner, homogeneous layer of the basement membrane has circular ridges which seem to fit into grooves on the bases of the epithelial elements.

In adult vertebrates the elements of the epithelium, especially of ectodermal and entodermal origin, and of the connective tissue are morphologically independent of one another and transformations between them are very rare.

Types of Epithelial Tissue. As different types of epithelium may arise from the same germ layer, as different germ layers may produce similar epithelial types, and as the physiologic rôle of certain epithelia has not been determined, the various types of epithelial tissue are best classified in terms of (*a*) the shape of the epithelial cells and (*b*) their arrangement in the epithelial sheet.

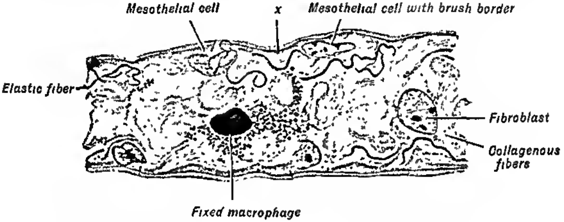

Fig 24 Cross section of a rabbit's mesentery; *x*, cross section of cell limits Iron-hematoxylin stain 750 ×. (A.A.M.)

The Shape of Epithelial Cells. Epithelial cells in the living condition change considerably in shape if the surface they cover is subject to stretching and contraction. Stretching flattens them; contraction permits them to gain in height. In the healing of wounds, in other pathologic conditions, and in tissue cultures, the form of an epithelial cell may change with its movement.

Squamous, cuboidal and columnar epithelial cells may be distinguished.

1. *Squamous Cells.* The height of the cell body is negligible in comparison with the other dimensions; the cell is a thin plate with regularly or irregularly outlined edges. In profile it looks like a slender rod which is usually slightly thickened in the vicinity of the nucleus (Figs. 23, 24).

2. *Cuboidal Cells.* The height is about equal to the width. When seen from the free surface they appear as small polygons; in a section perpendicular to the surface they have the form of squares. They are short prisms (Fig. 25).

3. *Columnar Cells.* The height greatly exceeds the width. In a section parallel to the surface the cells appear as small polygons (Figs. 26, 28, 29), in a perpendicular section as rectangles. They are irregular tall prisms. The end directed toward the underlying tissue is often tapering and pointed, or irregularly angular and branched. Cells of a fairly regular cylindrical shape are found in the epithelium of the higher sense organs. On curved surfaces the columnar and cuboidal cells assume the shape of truncated pyramids whose thin ends are directed to either the free or the fixed surface.

Transitional forms between these three types also occur. All three forms may be provided on their free surface with motile, hairlike outgrowths called *cilia* or with a *brush border* (p. 35).

Arrangement of the Cells in Epithelial Sheets. The cells of an epithelial sheet may be arranged in one or several layers. A sheet consisting of one layer of cells is called a *simple epithelium* of which

the following types are based on the shape of the cells: *simple squamous, simple cuboidal,* and *simple columnar epithelium.* The last type may have cilia on the free surface of the cells; it is then called *simple columnar ciliated* epithelium. All of the cells in a simple epithelium touch the underlying tissue. (See Figs. 25 to 29.)

When the epithelial sheet consists of several layers of cells it is called a *stratified epithelium.* Important in the mammals are the *stratified squamous* and *stratified columnar epithelium;* the latter at times may have cilia. A major example of the stratified columnar epithelium is the so-called "transitional" epithelium of the

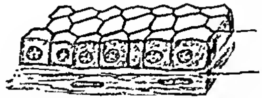

Fig. 25. Semischematic diagram of simple cuboidal epithelium, based on a section of the papilla of the kidney of a cat. Iron-hematoxylin stain. Redrawn and slightly modified after Stöhr-v. Möllendorff.

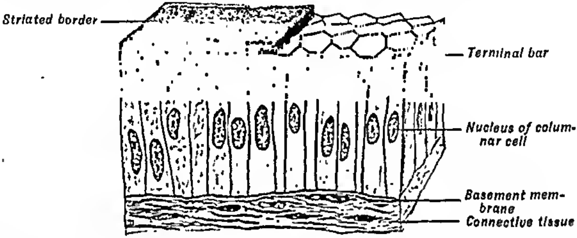

Fig. 26. Semischematic diagram of the columnar epithelium of the small intestine of a mouse. The striated border has been removed in the right half to show the network of terminal bars. Iron-hematoxylin stain. Redrawn and slightly modified after Stöhr-v. Möllendorff.

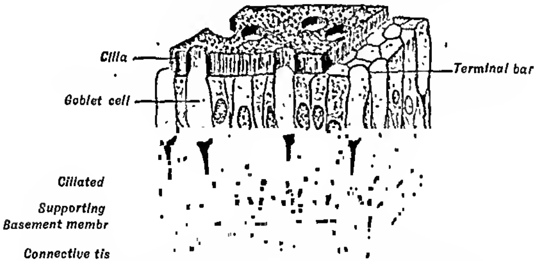

Fig. 27. Diagram of the arrangement of cells in the pseudostratified ciliated columnar epithelium. Redrawn and slightly modified after Stöhr-v. Möllendorff.

excretory passages leading from the kidney (see No. 10 which follows). In the stratified epithelia only the cells of the lowest layer touch the underlying tissue.

In the *pseudostratified columnar epithelium* (often provided with cilia) the cells seem to be arranged in more than one layer, but all of them touch the underlying tissue. (See Fig. 27.)

1. *Simple Squamous Epithelium.* Thin, platelike cells are arranged in one layer on the surface of the connective tissue and adhere closely to one another by their edges. On examination from the surface, especially after the cell limits are stained with silver nitrate, a typical mosaic pattern is seen. The individual cells have regular (usually hexagonal) or irregular, wavy outlines and each contains a nucleus (Fig. 23). In perpendicular sections a thin stripe is seen, subdivided into small parts which correspond to the single cells A given section will not pass through the nuclei of all the cells. In profile the contracted cell is a plump spindle (Fig. 24).

An epithelium of this variety is found in the human body on the inner surface of the wall of the membranous labyrinth and on the inner surface of the tympanic membrane of the ear; on the parietal layer of the capsule of Bowman, and in the descending limb of the loop of Henle in the kidney; in the rete testis, and in the smallest excretory ducts of many glands.

The mesothelium lining the serous cavities, the *mesenchymal epithelium* lining cavities in the connective tissue, and the *endothelial cells* lining the walls of the blood and lymph vessels—all three groups being squamous cells—are also considered by many authorities to be true epithelium. However, despite the structural similarity, these elements differ in origin and developmental potencies from true epithelium (p. 38).

2. *Simple Cuboidal Epithelium.* The low prismatic cell bodies adhere to one another by their lateral surfaces. On the free surface this epithelium appears as a mosaic of small, usually hexagonal polygons; the ribbon-like cross section of the sheet is subdivided into squares. (Fig. 25).

This epithelium is found in many glands, as in the thyroid, on the free surface of the ovary, on the chorioid plexus, on the inner surface of the capsule of the lens, in some areas of the labyrinth, in the excretory ducts of many glands and as the pigmented epithelium of the retina. The secreting epithelium in the terminal portions of many glands can often be placed in this class although the cells here usually have the form of truncated pyramids.

3. *Simple Columnar Epithelium.* The tall prismatic cells adhere to one another by their lateral surfaces. In sections parallel to the surface is seen a mosaic much like that in other simple epithelia (Fig. 26). In sections perpendicular to the surface the tall rectangles stand upright like fence palings. In many cases the oval nuclei are at approximately the same level.

Such an epithelium lines the surface of the digestive tract from the cardia to the anus and

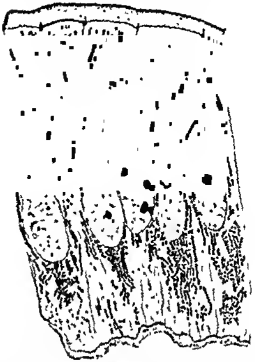

Fig. 28. Columnar epithelium from the intestine of a rat, showing striated border, terminal bars, and filamentous supranuclear, and granular infranuclear mitochondria. Iron-hematoxylin stain. 1000 ×. (A.A.M.)

is also common in the excretory ducts of many glands.

4. *Simple Columnar Ciliated Epithelium.* Like (3) above, except that the free surface of the cells is provided with cilia. Found in the uterus and oviducts, in the small bronchi, in some of the nasal sinuses, and in the central canal of the spinal cord.

5. *Stratified Squamous Epithelium.* The epithelial sheet is thick, and a perpendicular section shows the cells to be very unequal in form (Fig. 30). The layer next to the underlying tissue consists of cuboidal or even columnar cells, sometimes with rounded upper ends, as in the cornea. Then follow a varying number of layers of more or less irregular, polyhedral cells, often provided with excavations which fit the convex surfaces of

their neighbors, or with long stalks attached to the basement membrane. The nearer to the free surface, the more the cells are flattened. The superficial layers consist of thin, squamous cells.

This epithelium is found in the epidermis, the mouth, esophagus, a part of the epiglottis, a part of the conjunctiva, the cornea, the vagina, and a part of the female urethra.

6. *Stratified Columnar Epithelium.* The deeper layer or layers consist of small, irregularly polyhedral or fusiform cells which do not reach the free surface. The superficial cells are tall and prismatic and are not connected with the underlying tissue (Fig. 431) This epithelium is rare and covers small surfaces. It is found in the fornix of the conjunctiva, in the cavernous part of the urethra, in some places in the anal mucous membrane, in the pharynx, on the epiglottis, and in the large excretory ducts of some glands Some authors place the epithelium of the enamel organ in this group.

7. *Stratified Columnar Ciliated Epithelium* The cells are arranged as in the preceding type, but the free surface of the superficial columnar cells is provided with cilia (Fig. 32). It is found on the upper (nasal) surface of the soft palate, in the larynx and, transiently, in the fetal esophagus.

8. *Pseudostratified Columnar Epithelium.* In the pseudostratified epithelium the nuclei are at different levels and the cells lack uniformity (Fig. 27). Some of the cells, while attached to the underlying connective tissue, may lose their connection with the free surface. These substituting or supporting cells are covered by the tall, superficial cells. In a perpendicular section, the nuclei form several rows. Owing to mutual pressure, the cells may become very irregular in shape and the tissue may appear to be stratified. Such an epithelium occurs in the large excretory ducts of several glands (parotid) and in the male urethra.

9 *Pseudostratified Columnar Ciliated Epithelium* Exactly as in (8) except that the free surface of the cells is provided with motile or nonmotile cilia (Fig 27). Found on the greater part of the mucous membrane of the respiratory passages, in the eustachian tube, in a part of the

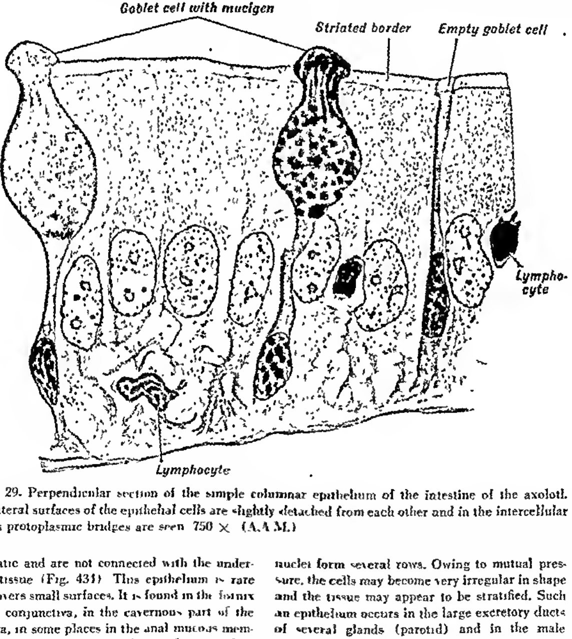

Fig. 29. Perpendicular section of the simple columnar epithelium of the intestine of the axolotl. The lateral surfaces of the epithelial cells are slightly detached from each other and in the intercellular spaces protoplasmic bridges are seen 750 × (A.A.M.)

tympanic cavity, in the lacrimal sac, and in the excretory passages of the male sexual system.

10. *Transitional Epithelium.* So called because it was supposed to represent a transition between the stratified squamous and the columnar epithelia. As it is found on the walls of hollow organs which are subject to great mechanical changes by contraction and stretching, its appearance varies greatly. In the contracted condition it consists of many cell layers. The deepest elements have a cuboidal or even columnar shape, then follow several layers of irregularly polyhedral cells, while the superficial layer consists of large cells with a convex free surface and excavated bevels fitting the subjacent polyhedral elements. In the stretched condition, usually only two layers can be distinguished: a superficial layer of large squamous elements over a layer of irregular, cuboidal cells. Some investigators believe that the superficial cells are connected with the underlying tissue by means of thin stalks and that this epithelium is accordingly of the simple type.

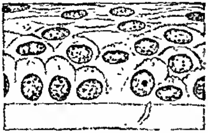

Fig. 30 Stratified squamous epithelium of the cornea of a monkey. Diplosomes above the nuclei. Redrawn after Zimmermann

The transitional epithelium is characteristic of the mucous membrane of the excretory passages of the urinary system from the renal calyces to the urethra.

The above classification applies only to the epithelia of the higher vertebrates. In chronic inflammatory irritations or in some neoplasms, one type of epithelium may change into another through the process of *metaplasia*. Thus, the columnar bronchial epithelium may change into the *stratified squamous*.

Inner Structure of Epithelial Cells. The nucleus is generally single and of simple shape. In the squamous cells it is an oval disk; in the cuboidal cells it is spherical; in columnar cells it extends along the cell axis and may appear cylindrical. Epithelial cells with several nuclei

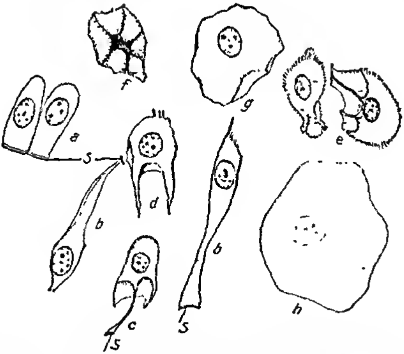

Fig. 31. Isolated cells from the stratified squamous epithelium of the cornea of the ox: *a*, Basal cells; *s*, cuticular border; *b*, club-shaped cells; *c*, cell with wing-shaped processes and with a thin stalk attached to the basement membrane; *d*, similar cell from one of the more superficial cell layers; *e*, prickle cells in profile; *f*, same in surface view; *g*, squamous cell; *h*, superficial flattened cell. 740 ×. After Schaffer.

sometimes occur (liver cells, parietal cells). The cell center, represented by a diplosome, is usually located above the nucleus and in columnar cells may occupy a position directly under the free surface.

Mitochondria are usually abundant. In squamous cells they surround the nucleus, in columnar cells they are arranged mainly above and below it. The longer mitochondria are usually parallel to the axis of the cell body. A *Golgi net* is always present, typically above the [illegible] it

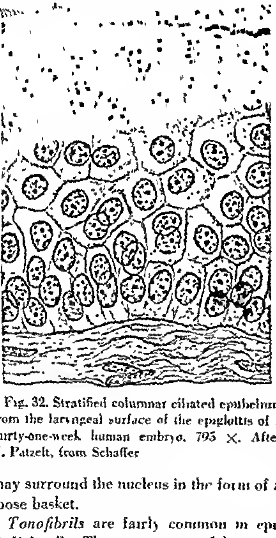

Fig. 32. Stratified columnar ciliated epithelium from the laryngeal surface of the epiglottis of a thirty-one-week human embryo. 795 ×. After V. Patzelt, from Schaffer

may surround the nucleus in the form of a loose basket.

Tonofibrils are fairly common in epithelial cells. They are supposed by some to give the soft cell body a rigid supporting framework, while others believe them to be only tension striae in the cytoplasm. They reach an especially high development in the stratified squamous epithelium where they form parallel or wavy bundles which pass from one cell body to another through the intercellular bridges.

A peculiarity of all epithelial cells is their *polarity*—the fact that the "proximal" side of the cell attached to the underlying tissue differs from the "distal" side, directed toward the free surface. The *cell axis*, or imaginary line connecting these two sides of the cell, is usually perpendicular to the surface of the epithelial sheet.

The polarity of epithelial cells results from their arrangement in sheets, for the two surfaces of a sheet of cells cannot have the same conditions of existence. The polarity manifests itself by the arrangement and the shape of the mitochondria at the base and at the top of glandular cells, by the unilateral position of the cytocentrum and the Golgi net, etc. Generally, the proximal end of an epithelial cell is less differentiated and therefore more similar in the various types of epithelium, reflecting the fact that the proximal end is mainly instrumental in the reception of the nutritive material, which must always be similar. The distal part, on the contrary, is subject to manifold external influences and in its protoplasm, above the nucleus, appear the different products of metabolism which are specific for each kind of epithelium—the secretory inclusions, the cuticular formations, etc.

The general structure of the epithelial elements just described presents innumerable variations due both to the special type of epithelium and to changing functional conditions. The details of these variations are described with the structure of the various organs in the chapters on special histology. All the types of inclusions mentioned in the introduction of this book may occur in and profoundly modify the structure of epithelial cells.

The Free Surface of Epithelial Cells. Structures may develop on the free surface of epithelial cells by (1) specific modification of the superficial layer of protoplasm and (2) formation of membrane-like products of secretion (cuticles).

1. ***Specialization of the Superficial Protoplasm.*** The simplest of the first group of structures is a thin layer of condensed protoplasm on the free surface. This is not a true membrane which can be isolated. In a perpendicular section it appears on the free edge as a thin refringent line. As an example, the superficial cells of the transitional epithelium of the urinary bladder show such a condensed cytoplasm at their free surface.

In all vertebrates the cells of the simple columnar intestinal epithelium have a distinct layer of modified protoplasm, the *striated border,* on their free surface. In a section the border shows a regular, fine, perpendicular striation, and is supposed to consist of minute protoplasmic processes bound together by a cement substance (Figs. 26, 28). Presumably, it plays an important rôle in the absorption of nutritive substances from the intestinal cavity.

A somewhat similar structure—a *brush border*—is found on the free surface of the epithelial cells of the main segment of the uriniferous tubules of the kidney (Fig. 425). This structure seems to change considerably according to the functional condition of the tubules. It consists of nonmotile, hairlike outgrowths which stand upright in the fashion of a dense brush and are provided at their base with small granules. The brush border is supposed to be instrumental in the absorptive activity of the epithelium. A similar brush border has been described on the surface of the mesothelium (Fig. 24) and on the epithelium of the placental villi.

The highest structural differentiation of the free surface of the epithelium is reached in the *ciliated cells* (Fig 32). These carry on their free surface a large number of cilia, *i. e.,* thin, usually motile, processes. The length of the cilia in different types of epithelium varies considerably. Their substance is homogeneous, although an axial filament or a cross striation has sometimes been described. As a rule, at the base of each cilium, in the superficial layer of protoplasm is a small thickening, the *basal corpuscle.* It has a high refractive index and stains black with iron hematoxylin.

The movement of the cilia, which propels a constant stream of mucus or other liquid secretory material, consists of a rapid effective beat and a slower recovery stroke, always in one direction. The beat of each cilium begins slightly later than the beat of the one which precedes it in the direction from which movement proceeds, so that the beating moves across the ciliated surface in rapid and regular waves. If the connection between the cells in the path of the movement is severed, the

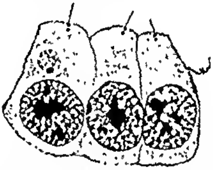

Fig. 33. Three epithelial cells with central flagella from a urinary tubule of the mesonephros of a rabbit embryo of 9.5 mm. 1500 ×. (A.A.M.)

waves in the separated areas become independent. The basis of the ciliary movement is not known; it is probably much the same as that in the muscle cell.

In the respiratory passages the ciliary movement drives the mucus toward the mouth and with it are eliminated the particles of inhaled dust which stick to the mucous membrane. In the ductuli efferentes of the testis the ciliary movement probably helps to forward the spermia from the rete testis to the duct of the epididymis.

In the epididymis the epithelium carries long, nonmotile cilia which are supposed to help in the elimination of the secretion from the cells.

The nonmotile hairs of the epithelium of the maculae and the cristae in the inner ear serve as receptors of vibratory stimuli and transmit them to the cell body.

In the same category of structures are the *central flagella,* which have been found in many

sometimes occur (liver cells, parietal cells). The cell center, represented by a diplosome, is usually located above the nucleus and in columnar cells may occupy a position directly under the free surface.

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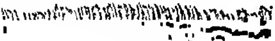

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uterine mucous membrane at the beginning of pregnancy and in some areas of the ectoderm of the embryo (trophoblast).

Terminal Bars. In various kinds of epithelium stained with iron hematoxylin. the free surfaces of the single cells are outlined by black lines different from those appearing after the action of silver nitrate. They are smoothly outlined rods of a dense cement substance which solders the edges of the cell surfaces, and are called *terminal bars* (Fig. 25) because they are supposed to close the intercellular spaces on the free surface.

The terminal bars, when stained with iron hematoxylin, appear as dots if they are seen in cross section, or as short black lines if they happen to lie in the plane of the section (Fig. 34). If the free surface of the cells is provided with a cuticle, a striated or brush border, or with cilia, the bars are always located beneath these structures.

Blood Vessels. As a rule, epithelial tissue lacks blood vessels. The nutritive liquid from the blood vessels of the underlying connective tissue reaches the epithelial elements after passing through the basement membrane and through the thin intercellular spaces between the epithelial cells. If the epithelium forms a very thick layer, as in the skin, the surface of the connective tissue is usually provided with outgrowths, *papillae*, which carry blood capillaries, bulge deeply into the epithelium and probably facilitate nutrition. In a few cases (stria vascularis of the cochlea) loops of blood capillaries with thin strands of connective tissue may penetrate the epithelium

Extraneous Cells. Foreign cells may enter the epithelium from the connective tissue. Nearly everywhere the epithelium is provided with numerous terminal branchings of nerve fibers which pierce the basement membrane and run between and even into the epithelial cells The epithelium of the intestine is always infiltrated in special areas by a multitude of lymphocytes; these may even push aside and disfigure the epithelial cells. After they have left, the latter regain their usual form and resume their former relations. This infiltration reaches its highest degree in the thymus.

Regeneration of Epithelium. The epithelial layers, especially those which cover the outer surface of the body and the intestinal tract, are subject to constant mechanical and other injuries. Under physiologic conditions their cells perish continuously and are shed. This is especially manifest in the epidermis where the superficial cells are continuously undergoing a peculiar degeneration, called *cornification*. The cornified cells are constantly desquamated and are replaced by new ones which arise through the transformation of the cells of the deeper layers. In the respiratory passages, in the inner cavities of the body, and especially in most of the glands, where the epithelium is not accessible to external noxious agents, degeneration is rare.

The physiologic loss of cells in the epithelium is balanced by a corresponding regeneration.

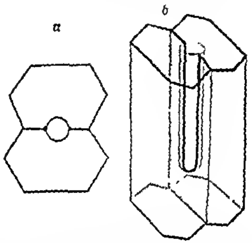

Fig. 34. Diagram of two adjacent glandular cells with a secretory capillary between them and with terminal bars: *a*, View in cross section; *b*, side view. Redrawn after Zimmermann.

In vertebrates this is always effected through mitotic proliferation of indifferent epithelial elements. In the stratified squamous epithelium the mitoses are found mainly in the deeper columnar and polyhedral cell layers (stratum germinativum) The simple columnar epithelium of the stomach and the intestine is regenerated from special areas of proliferating undifferentiated epithelium which are located in the base of the gastric foveolae (Chapter XVIII) or in the crypts of Lieberkuhn. In the pseudostratified and stratified columnar epithelium, dividing cells are occasionally found between the resting ones. Their round, contracted bodies usually move toward the surface. Whether the small, deeper cells connected with the underlying tissue, which were supposed to be the source of regeneration in the pseudostratified epithelium and were correspondingly called "substituting cells," really play this rôle, seems doubtful.

epithelial cells, especially in glandular ducts (Fig. 33) and in the mesothelial cells in the serous membranes. At its free surface the cytoplasm contains a diplosome, with its axis standing perpendicularly. From the distal centriole an extremely thin, apparently nonmotile filament extends beyond the cell body.

2. *Cuticles.*—Cuticle is generally a layer of a more or less solid substance which is secreted by and covers the free surface of an epithelial sheet. Unlike the protoplasmic crust, the cuticle is sharply delimited from the cell surface and can be detached from it. The cuticle often becomes impregnated with lime salts, chitin, etc., and may consist of separate areas, each corresponding to one cell (tooth enamel), or it may become continuous through fusion of contiguous areas.

In the mammals the cuticles are infrequent; examples are the enamel of the teeth; the capsule of the lens; and the tectorial membrane, the lamina reticularis, and the otolithic membrane in the internal ear.

The Connections Between the Cells in an Epithelial Sheet. Adjacent epithelial cells cohere so tightly that relatively very strong mechanical forces are necessary to separate them. This cohesion is particularly striking in the epithelium of the oral cavity and of the intestinal tract where the movement of hard masses is unable to separate the cells from one another. It is probable that the small amount of interstitial substance acts as a plastic cement. Microdissection studies have shown that in most types of epithelium the superficial cytoplasm of the living cells contains an adhesive substance which keeps the cells together. It is possible that adjacent cells might adhere through interaction of the fibrous protein molecules in their surface membranes. This question is being actively investigated with physical and chemical methods.

According to some, the epithelial cells are kept together by many small, protoplasmic processes, running from one cell body to another and forming intercellular bridges. If the epithelial cells are isolated from one another, the broken bridges appear as short, thornlike outgrowths on the surface of the cell body. These bridges are most conspicuous in the stratified squamous epithelium of the skin, the epidermis. Here the deeper cells are separated from one another by clear, intercellular clefts filled with liquid. The intercellular bridges cross the clefts and each shows at its middle a small thickening, the *bridge corpuscle* or *desmosome*. The tonofibrils, which reach a high grade of development in these cells, run from cell to cell through the intercellular bridges (Fig. 281).

In those epithelia which have no intercellular spaces, the lateral surfaces of the cells adhere tightly to one another and intercellular bridges cannot be seen. In the simple columnar epithelium the cells sometimes separate and then bridges are distinct. Because this separation can be produced by the use of fixing reagents which cause shrinkage, these bridges are believed by some to be artefacts.

After the action of silver nitrate, especially in the simple squamous epithelium (or mesothelium and endothelium), the cell limits are outlined by black contours. What look to be large openings in such preparations, the "stomata" which the old histologists thought existed between the edges of the simple squamous epithelial (mesothelial or endothelial) cells, are artefacts. They are sometimes very prominent as black dots along the intercellular lines.

Epithelial cells sometimes cohere intimately by means of special structures. Thus, in the convoluted uriniferous tubules the lateral surfaces of the cells are provided with alternating ridges and grooves which interdigitate with those of neighboring cells (Fig. 427). When the limits between the cells cannot be detected the epithelial sheet has the character of a *syncytium*. This is found, for instance, in the epithelium of the

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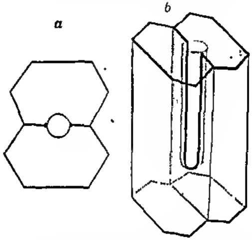

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3. *Mesenchymal epithelium* is the simple layer of squamous cells which lines the subdural and subarachnoid spaces, the perilymphatic spaces in the inner ear and the chambers of the eyeball (The cavities of joints are *not* lined by mesenchymal epithelium. See p. 153.)

These elements seem to originate simply through flattening of common fibroblasts. The relations between the mesenchymal epithelium and the mesothelium have not been sufficiently elucidated.

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In pathologic conditions, as after local injuries, almost every type of epithelium in the human organism may display a considerable ability for regenerative proliferation, although the process may produce new, abnormal cell types. In tissue cultures several types of epithelium (thyroid, iris, and liver) were observed to proliferate and to grow in "pure cultures" of thin sheets of flattened cells which did not change for some months. The various types of epithelium, especially those of ectodermal and entodermal origin, seem to keep their specific character even after a prolonged series of transplantations. Although some workers have claimed that epithelial cells in tissue culture develop into fibroblasts, the evidence to date indicates that this is not true.

The proliferation in epithelial regeneration occurs only by mitotic division. Amitosis has been found in the transitional epithelium of the urinary bladder, in the mesothelium of the serous membranes and especially in tissue cultures, but it seems to result only in the formation of multinucleated cells.

In the body, excluding ciliated cells, the epithelial elements are not motile, as a rule. In the healing of wounds and in tissue cultures, however, they are seen to flatten and to display a peculiar gliding movement, by which they rapidly cover large denuded areas of connective tissue before regenerative division sets in. Occasionally, epithelial cells have even been seen to form ameboid pseudopodia. Wolbach and collaborators have shown that a deficiency of vitamin A in the diet of guinea pigs and rats results in atrophy of most of the epithelia of the body; in their place appears "a stratified keratinizing epithelium, identical in appearance in all locations, and arising from focal proliferation of basal cells." When the vitamin is supplied, the original type of epithelium appears in a few days. (See also Arey, 1936.)

Endothelium, Mesothelium, and Mesenchymal Epithelium. The term "endothelium" has been used in different senses. Originally it was chosen for those simple layers of squamous cells which arise from the mesoderm and line cavities of the body which do not open on its surface. These included the serous cavities, the blood and lymph vessels, the anterior chamber of the eye, etc. Histologically, no difference can be found between the elements just mentioned and the squamous cells of obviously ectodermal or entodermal origin, as the simple squamous epithelium of the labyrinth. All such layers of squamous cells, when treated with silver nitrate, will show the typical mosaic of black cell limits (Fig. 23). Therefore, many authors designate as epithelium each continuous cell layer covering an outer or an inner surface in the body.

The structure of the cells in their fully developed physiologic condition cannot, however, serve as the only criterion for their classification. Squamous cells arranged in a single layer, although having the same structure, may have a totally different embryonic origin, and quite different developmental potencies. On the basis of these criteria, besides the true simple squamous epithelium which originates from either ectoderm or entoderm, and is always sharply separated from the connective tissue elements, three types of a histologically similar tissue which originate from the mesoderm proper or from its derivative, the mesenchyme, can be distinguished: (1) the endothelium, (2) the mesothelium, and (3) the mesenchymal epithelium.

1. The name *endothelium* should be reserved for the simple layer of squamous cells which lines the inner surface of the wall of the blood and lymph vessels and of the heart (p. 230). In the early stages of embryonic development the endothelium arises through the flattening of mesenchymal cells (p. 100). In the later ontogenetic stages and in the adult it seems to be a highly differentiated tissue which grows only through proliferation of its own elements and not through transformation of other connective tissue cells. On the other hand, endothelial cells can be transformed into fibroblasts.

2. The *mesothelium* is a simple, squamous cell layer which covers the surface of all the serous membranes (peritoneum, pericardium, pleura). Its elements have the classical structure of true squamous epithelial cells (Fig. 23). The surface of their protoplasm is provided with a thin, condensed, crustlike layer which carries, sometimes, a more or less distinct brush border (Fig. 24). The surface of the flattened, round or oval nucleus is slightly excavated and here there is a diplosome with a typical central flagellum protruding into the serous cavity.

The prospective potencies of these elements are of double nature—epithelial and fibroblastic. In tissue cultures the mesothelium of mammals may show for a time a purely epithelial type of growth in islands and sheets of polyhedral, flattened cells. Tumors of epithelial character may develop from the mesothelium and, possibly, structures similar to uterine glands. On the other hand, in inflammation, the mesothelial cells, after a period of contraction and of rounding off, finally give rise to typical fibroblasts, *i. e.*, to connective tissue cells. The same occurs in tissue cultures. They are never transformed into ameboid phagocytes.

pended in the plasma are several kinds of formed elements: the *red corpuscles* or *erythrocytes,* colorless corpuscles, the so-called *white blood corpuscles* or *leukocytes,* and in mammals, the *blood platelets.*

RED BLOOD CORPUSCLES. ERYTHROCYTES

In the mammals the erythrocytes are nonmotile, highly differentiated cells which have lost their nucleus, Golgi net, mitochondria and centrioles during maturation. In all the other veterbrates they retain the nucleus.

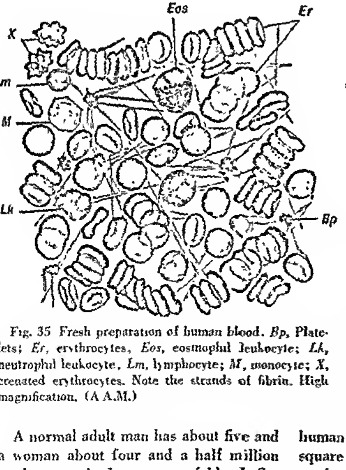

Fig. 35 Fresh preparation of human blood. *Bp,* Platelets; *Er,* erythrocytes, *Eos,* eosinophil leukocyte; *Lk,* neutrophil leukocyte, *Lm,* lymphocyte; *M,* monocyte; *X,* crenated erythrocytes. Note the strands of fibrin. High magnification. (A A.M.)

in man the diameter averages 7.74 μ, the thickness at the edge, 1.9 μ. According to some estimates, the erythrocytes of man have a diameter well over 8 μ and the smaller figure given here is due to dehydration during preparation. The total surface area of all the erythrocytes in the

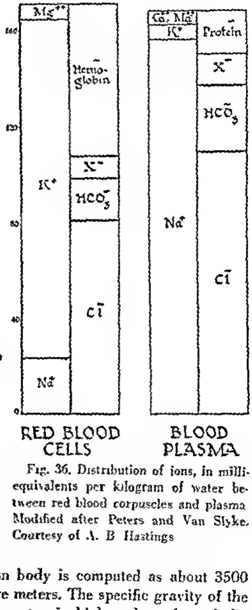

Fig. 36. Distribution of ions, in milliequivalents per kilogram of water between red blood corpuscles and plasma Modified after Peters and Van Slyke. Courtesy of A. B Hastings

A normal adult man has about five and a woman about four and a half million erythrocytes in 1 cu. mm. of blood. Sojourn in high altitudes causes a marked increase in their number. The changes in pathologic conditions are still more prominent. The erythrocytes are not always evenly distributed over the circulatory system.

The size of the erythrocytes, under normal conditions, is remarkably uniform; human body is computed as about 3500 square meters. The specific gravity of the erythrocytes is higher than that of the plasma; the estimations vary from 1.02 to 1.08.

The color of the erythrocytes is a pale, greenish yellow. This is especially marked at the periphery of the corpuscle where the layer of the colored substance is thickest. In dense masses of erythrocytes the yellow color turns into a distinct red.

Chapter III

BLOOD

THE embryonic connective tissue, the *mesenchyme*, gives rise to the blood, the blood vessels, and the various types of connective tissue. The mesenchyme develops from the mesoderm immediately after the formation of the germ layers and soon accumulates in masses between them. The mesenchymal cells at first are irregularly stellate and connected by their processes. They undergo many changes to form the various blood and connective tissue cells.

There are four main types of connective tissue, all characterized by an abundant intercellular substance: (1) blood and lymph, (2) connective tissue proper, (3) cartilage and (4) bone. In the blood and lymph the intercellular substance is liquid. In the connective tissue proper, of which there are many types, the intercellular substance always contains fibers and varies from a soft jelly to a tough fibrous mass. In cartilage the intercellular substance contains masked fibers and has a rubbery consistency. In bone, the fibrous intercellular substance is impregnated with lime salts.

In the adult organism the various tissues of the connective substance cannot be sharply separated from one another in all respects. The fibers of the connective tissue proper continue into both cartilage and bone, and certain characteristic chemical substances are common to the intercellular substance in the connective tissue proper, cartilage, and bone. Similarly, the cells of the blood cannot be separated from those of the connective tissue proper, as there is a constant exchange of cells between them. Certain cells of the blood and the other connective tissues may display marked differences in form and function when their environment is changed. Thus, a leukocyte which seems to be inactive while in the blood may previously have been very active while in the connective tissue proper and may become active again on reentering this tissue from the blood.

In the earliest stages of development, the endothelial cells of the blood vessels and the blood cells arise simultaneously from the same mesenchymal elements. Moreover, the embryonic endothelium occasionally turns into blood cells. In the later stages, however, the endothelium becomes more differentiated and independent, and new vessels arise only through sprouting of preexisting ones. Thus all of the vessels, including the heart, become a comprehensive specialized system, described in Chapter X. The blood vessels are always accompanied by connective tissue.

THE FORMED ELEMENTS OF THE BLOOD

The blood of adult vertebrates is a red liquid which circulates in a closed system of tubes, the blood vessels. Its total quantity in man is estimated at about 7 per cent of the body weight. The liquid menstruum of the blood, the *plasma*, appears colorless in a thin film examined under the microscope, but varies from gray to yellow, according to species, when seen in large amounts with the naked eye. Sus-

pended in the plasma are several kinds of formed elements: the *red corpuscles* or *erythrocytes,* colorless corpuscles, the so-called *white blood corpuscles* or *leukocytes,* and in mammals, the *blood platelets.*

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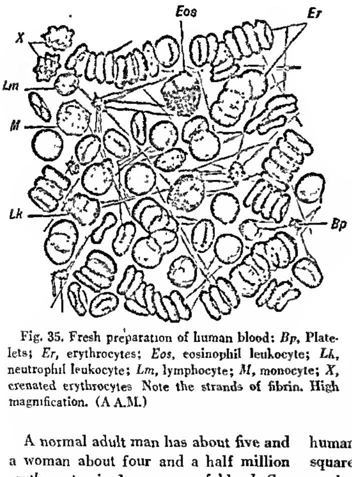

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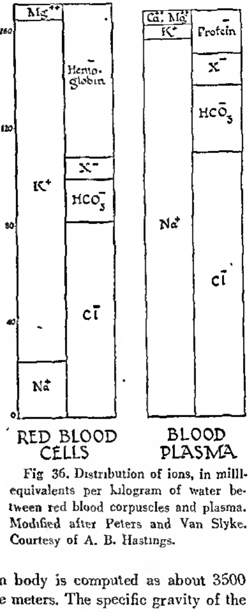

Fig 36. Distribution of ions, in milli-equivalents per kilogram of water between red blood corpuscles and plasma. Modified after Peters and Van Slyke. Courtesy of A. B. Hastings.

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The color of the erythrocytes is a pale, greenish yellow. This is especially marked at the periphery of the corpuscle where the layer of the colored substance is thickest. In dense masses of erythrocytes the yellow color turns into a distinct red.

The pigment which gives the erythrocytes their color can be easily separated from the corpuscles. It then dissolves in the plasma and gives it a distinct color while the corpuscles become colorless, although they keep their form more or less. This process is called *hemolysis.* The pigment is *hemoglobin;* the colorless part which remains after the hemoglobin leaves is called the "ghost," "the shadow" or the *stroma.* Hemoglobin and the colorless substances are present in the erythrocytes as an optically negative, structureless, colloidal mixture of which hemoglobin forms about 95 per cent of the dried weight.

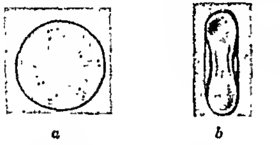

Fig. 37. Red blood corpuscle in 0.9 per cent NaCl solution, seen from the broad surface (*a*) and in profile (b); thorn apple form of a red blood corpuscle after treatment first with 0.3 per cent NaCl and then with 0.9 per cent NaCl (*c*). Mulberry form of red corpuscle after treatment with 1.5 per cent NaCl solution seen from the surface (*d*). After Broderson

The erythrocytes of the mammals are biconcave disks. In profile they have elongated bodies with rounded ends and a constricted middle part, more marked on one side than on the other (Fig. 35).

Some investigators claim that they are shallow cups, that is, convex-concave disks, and that the biconcave form is merely the result of shrinkage due to an increase in the osmotic pressure of the plasma during examination. It is possible that in the normal blood both forms, as well as all transitions between them, exist at the same time. In the camel and the llama the erythrocytes are biconcave ovals.

The erythrocytes are extremely soft and flexible. The slightest mechanical influences distort them, but the usual form is at once restored as soon as the mechanical factor ceases to act. This can be seen easily during the observation of the circulating blood in living capillaries. When an erythrocyte is forced through a blood vessel of very small caliber it becomes considerably drawn out, but resumes the disk shape as soon as it enters a larger vessel. In living condition their substance appears homogeneous even with dark-field illumination. Ultracentrifuged erythrocytes are stratified into two or three layers; this indicates that they are composed of at least three substances (Beams).

Under physiologic conditions the interior of the erythrocytes and the plasma are in a state of osmotic equilibrium. If the molecular concentration of the plasma is lowered through addition of water, water enters the erythrocyte. If the osmotic pressure of the plasma increases, the interior of the erythrocyte gives up water to the plasma (*crenation*, Figs. 35, 37). A solution of 0.9 per cent sodium chloride is isotonic with normal human plasma and therefore does not alter the size or form of the erythrocytes; it is called physiologic salt solution.

A characteristic physical property of the erythrocytes is their marked tendency to adhere to one another by their broad surfaces and to assemble in long, curved, sometimes branching columns resembling piles of coins (Fig. 38). This can be observed in a simple preparation of a drop of fresh, undiluted blood. The piles or *rouleaux* arise at once after the currents of the liquid in the drop have ceased; a

slight pressure on the coverslip breaks them up. They are seen to be formed even in the living body while circulating in the blood vessels. The adhesion is so strong that, if the motion of the blood stream in the vessel is not too swift, the piles are seen gliding, serpent-like, through the smaller vessels. The cause of the rouleaux formation is not known. Some believe that it is a display of surface tension forces which cause bodies suspended in a fluid to apply to one another by their greatest surfaces.

Irregular, persisting clumps of erythrocytes occur in the circulating blood in a variety of pathological conditions; these masses of erythrocytes have been called *sludges* and have been studied extensively by Knisely and co-workers. They may cause severe local or generalized damage.

Agglutination. Various reagents, as acid salt solutions or solutions of glucose, may cause agglutination of erythrocytes. They attract one another and assemble in small, dense groups in which they assume a polyhedral form through mutual pressure. In some pathologic or experimental conditions, agglutination of erythrocytes under the influence of agglutinins can occur in the circulatory system of the living organism; the resulting clusters of erythrocytes obstruct the small blood vessels and may lead to severe injuries of the tissues involved.

Abnormal Forms of the Erythrocyte. A detailed description of abnormal forms of erythrocytes belongs to the textbooks of pathology. Sometimes, however, they occur even in the blood of an apparently normal individual, or they appear as a manifestation of an accelerated formation of blood They must, therefore, be mentioned in this place.

The erythrocyte may be unusually large, or, on the contrary, quite small. This phenomenon is called *anisocytosis*. The largest corpuscles are called *macrocytes*, the smallest ones, *microcytes*. If the erythrocytes acquire irregular contours, *poikilocytosis* is present. This phenomenon occurs in the human blood when there is a grave disturbance of hemopoiesis as in pernicious anemia. In a special type of anemia the erythrocytes are *sickle-* or *crescent-shaped*.

The hemoglobin content may show great variations: in chlorosis each corpuscle contains an abnormally small quantity of this pigment and consequently appears paler than usual (*hypochromic*). In pernicious anemia the individual erythrocytes are, on the contrary, abnormally rich in hemoglobin (*hyperchromic*).

The normal, mature erythrocytes in a dry smear are *acidophil;* that is, in a mixture of a red acid (eosin) and a blue basic (methylene blue) dye (Romanowsky mixture) they are stained electively with the acid dye and appear red (Fig. 39). In some cases, however, their substance will stain purple or bluish with this mixture This condition is called *polychromia*. Polychromatic erythrocytes may occur in the human blood in various anemias. Under physiologic conditions they are rarely found in the circulating blood, but are quite common in the bone marrow. In the embryo the majority of the circulating erythrocytes are polychromatic. The young forms of the erythrocytes (*erythroblasts*) also have a polychromatic protoplasm. Thus, polychromia is a manifestation of immaturity of the red blood corpuscle, although

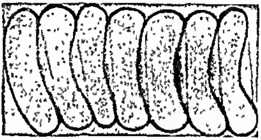

Fig. 38. Rouleaux formation of human erythrocytes in a fresh blood preparation. After Broderson.

in some cases it may also be found in degenerating erythrocytes. The polychromatic erythrocyte always shows a "reticulated substance" on supravital staining. It is possible that this stained network is the result of an artificial clumping of the diffused substance which causes the polychromatophilia seen with the Romanowsky stain. Protoporphyrin has been found in the blood and occurs only in the *reticulocytes;* it is possibly the substance which is stained with brilliant cresyl blue in these almost mature erythrocytes. The term "reticulocytes" is a poor one because of the frequent confusion with "reticular cells."

In the case of *basophil granulated* or *"stippled" erythrocytes*, with the Romanowsky stain, the substance of the erythrocytes is mottled with numerous, fine, basophil, blue granules. These granules seem to exist as corpuscular elements in the living erythrocyte. The stippled erythrocytes occur in the blood of embryos and of adult mammals including man They are found in various types of anemia, especially those of toxic nature, as in lead poisoning. In the hemopoietic organs of the adult

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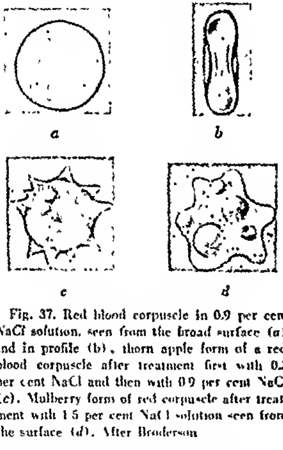

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they do not occur under physiologic conditions. It is possible that the stippled condition is also a manifestation of immaturity in much the same way as the polychromia; but of an immaturity which is physiologic only in the embryo, while in the adult it is an indication of an abnormal return to an embryonic type of development. The origin of the basophil granules is traced to either the nucleus of the erythroblast or to its basophil cytoplasm. It is possible that their substance is similar to the basophil substance in the polychromatic erythrocyte and differs from it only by its granular instead of diffuse distribution in the corpuscle. From this point of view the granules would be a manifestation of a degenerative change of the young erythrocytes

Quite different from the basophil granules of the mottled erythrocytes are the peculiar granular *Howell-Jolly bodies*. These are undoubtedly remnants of the nuclear chromatin. They often occur in seemingly quite normal erythrocytes of adult and especially of embryonic mammals. They are small, sharply outlined, round or angular bodies *which stain very intensely with* nuclear dyes, and especially with methyl green, which never stains the basophil granulation. Their number is limited to one or two in an erythrocyte.

In some pathologic cases the erythrocytes contain the *rings of Cabot*. Although some believe them to be the membrane of the dissolved or extruded nucleus, Schleicher holds that they are artefacts which represent aggregated and denatured proteins of erythrocytes exposed to hemolytic agents.

Certain toxins cause the appearance of peculiar granules in the erythrocytes of the circulating blood—the *granules of Ehrlich-Heinz*. They can be stained with acid dyes in fixed smears and are products of the disintegration of hemoglobin.

Function of the Erythrocytes. The erythrocytes are carriers of oxygen. In the blood vessels of the lungs (or gills) their hemoglobin combines with oxygen and is transformed into oxyhemoglobin. In the tissues of the body where the oxygen tension is much less than in the respiratory organs, oxyhemoglobin is reduced and its oxygen is used in the metabolic processes of the cells. Hemoglobin plays an equally important part in the transport of carbon dioxide from the tissues to the lungs. As it loses oxygen it becomes a weaker acid, the diminution in its acid strength being nearly sufficient to compensate for the carbonic acid formed from the oxygen it delivers to the tissues. In the lungs, as oxygen is taken up and carbon dioxide is lost, it again becomes a stronger acid. In addition, part of the carbon dioxide carried from the tissues to the lungs is combined directly with hemoglobin in the form of hemoglobin carbamate.

THE COLORLESS CORPUSCLES; LEUKOCYTES OR WHITE BLOOD CORPUSCLES

The blood of all animals contains a number of colorless corpuscles. Although the histogenesis and morphology of these leukocytes have been studied intensively, little is known of their fundamental physiologic functions.

The white blood corpuscles are more resistant to change in the surrounding medium than the erythrocytes. In a drop of fresh blood, if desiccation is prevented, they remain alive for a considerable time and can be studied easily. Their number is far smaller than that of the erythrocytes, averaging in the normal human blood 5000 to 9000 in a cu. mm. In children the figures are higher. The number of leukocytes in the circulating blood varies at different times of the day, during digestion, in the various parts of the circulatory system and, in addition, may change rapidly under the influence of numerous conditions which are hard to control. Consequently, many of the leukocyte counts that are made so frequently in the clinic have only a relative value. The number of circulating non-granular leukocytes seems to be partially under control of pituitary and adrenal cortical hormones.

C: 1, Myeloblast with azure granules from normal human bone marrow; *2*, myeloblast from the blood in chronic myeloid leukemia; *3, 4*, lymphoblasts from the blood in chronic lymphatic leukemia; *5*, lymphoblast from the blood in subacute lymphatic leukemia. (Cells *2–5* are from pathologic human blood.) May-Grünwald-Giemsa-stained dry smears. After Downey.

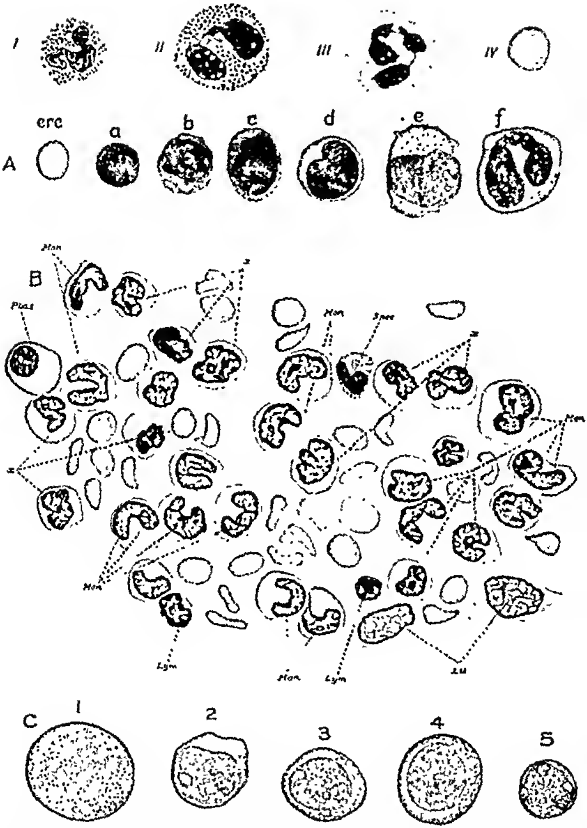

Fig. 39. *I*, Basophil leukocyte; *II*, eosinophil leukocyte; *III*, neutrophil (heterophil) leukocyte; *IV*, erythrocyte, from human blood stained with May-Grunwald Giemsa. *I*, stained with thionin (Fig. 73). (A A.M.)

A· *erc*, Erythrocyte; lymphocytes (*a*, *b*, *c*, *d*), monocytes (*e*, *f*) from a Romanowsky-stained, dry smear of human blood. (A.A.M.)

B· Section through a splenic *sinus* of a rabbit infected with *Bacterium monocytogenes*: *Mon*, Monocytes; *Lym*, lymphocytes; *x*, transitions from lymphocytes to monocytes; *Plas*, plasma cell; *Lit*, lining (littoral) cells; *Spec*, heterophil leukocyte. Hematoxylin eosin-azure stain, 1200 ×. After Bloom.

chondria are very scarce and small and have the form of small dots or very short rods. They are easily stained supravitally with Janus green (Fig. 40). Supravital staining with neutral red seldom reveals more than three to five inclusions in man. In many lymphocytes in some animals, as the rat, a sometimes considerable number of small red vacuoles can be seen around the cytocentrum. Except for occasional small lipoid droplets, no other inclusions are found in the living, unchanged lymphocytes.

Although the lymphocytes are nongranular leukocytes, Romanowsky-stained, dry smears occasionally reveal a few round granules of different sizes and of a bright purple color in their cytoplasm (Fig. 39, *A*). These are called *azurophil granules.* Unlike the granules of the granulocytes, they are not a constant, specific feature of this cell type.

In the guinea pig many lymphocytes and monocytes contain a large spherical inclusion, the *Kurloff body.* In dry smears it stains in the same way as do the azurophil granules and is probably also an accumulation of a substance elaborated by the protoplasm, although it has other staining reactions which differentiate it from the azurophil granules. In living cells the Kurloff body is a homogeneous, yellowish-green body.

Monocytes. There is much confusion as to just what a monocyte is, since the delimitation of this cell type has been obscured by the contradictory opinions of the proponents of the various theories of blood formation. When preparations of blood are examined objectively, it is seen that the nongranular leukocytes consist of a series of transition forms which begins with the smaller lymphocytes and ends with larger cells of quite different appearance, the monocytes, which will be described shortly. But in the midportion of this series of transitions is a group of cells which cannot be classified as either typical lymphocytes or typical monocytes. The following description refers to the typical monocytes of the blood; a discussion of their origin is found on p. 000.

The typical monocytes are large cells, 9 to 12 μ in diameter. In dry smears, where they are flattened and stretched, their diameter may reach 20 μ (Fig. 39, *A: c, f*). They constitute 3 to 8 per cent of the leukocytes of the circulating blood. Their enumeration is especially difficult because, as mentioned above, they cannot always be sharply differentiated from the larger lymphocytes of the blood.

In the typical monocytes, the cytoplasm is far more abundant than in the lymphocytes while the nucleus is relatively small (Fig. 39, *B*). In the older monocytes the nucleus has an eccentric position, and is oval or kidney-shaped. A few monocytes have a horseshoe-shaped or deeply constricted nucleus—these are the oldest ones. The nuclear membrane is much thinner and the chromatin granules finer and more numerous than in the lymphocytes. Therefore, the nucleus stains paler, especially in dry smears. One or two small nucleoli are always present, although not seen in dry smears.

The abundant cytoplasm has a pale, grayish-blue color in dry smears stained with eosin-methylene-azure. Special methods show that it contains the usual diplosome and a considerable number of mitochondria. At the periphery, near the indentation of the nucleus, is a Golgi net. Supravital staining with Janus green and neutral red reveals a spherical group of fine red vacuoles, the rosette, which surrounds the cytocentrum; its position corresponds with that of the Golgi net; the bluish-green stained mitochondria are arranged in a wreath around the rosette. The claim that this rosette is specific for monocytes has been shown to be incorrect, for other closely allied cell types may have neutral red rosettes (lymphocytes in the rat, plasma cells, some macrophages, some of the septal cells of the lung). The number of circulating monocytes may be in-

The leukocytes are true cells with a nucleus and cyptoplasm; they are all more or less ameboid. In fresh human blood several types can be distinguished: (1) Small cells, about the size of an erythrocyte or slightly larger, with a scanty, clear, homogeneous cytoplasm and a faintly outlined, relatively large round nucleus (*lymphocytes*); these merge by a series of transition forms into (2) slightly larger

Fig. 40. Lymphocyte of human blood showing mitochondria stained supravitally with Janus green. After Cowdry.

cells with an oval or indented nucleus and somewhat greater amounts of cytoplasm (*monocytes*); these cells are easily distinguishable from (3) cells with a cytoplasm filled with fine granules and a lobated nucleus (*heterophil granular leukocytes* [*neutrophil* in man]). (4) There are also a few cells with coarse, round, yellowish, brilliant granules and usually two clear spots representing the nucleus (eosinophil granular leukocytes). (5) Another type of leukocyte, the *basophil granular leukocyte*, is hard to identify when unstained. Thus, the white blood corpuscles may be separated into two groups: I. Nongranular leukocytes, and II. Granular leukocytes.

The Nongranular or Lymphoid Leukocytes; Agranulocytes. This group contains the lymphocytes and the monocytes. As we shall see in the section on the lymphatic tissue, the larger lymphocytes do not gain access to the blood stream under normal conditions and the large lymphocyte of the blood is the same as the medium sized lymphocyte of the lymphatic tissue. Under abnormal conditions, large lymphocytes of lymphatic tissue appear in the blood; they are usually called *lymphoblasts*.

The Lymphocytes. In human blood the lymphocytes are spherical cells 6 to 8 μ in diameter, although a few of them may be even a little larger. On the average they are slightly larger than erythrocytes.

The characteristic feature of a small lymphocyte is a relatively very large nucleus surrounded by a thin layer of cytoplasm. The nucleus is spherical; on one side it always has a more or less marked *indentation*. In stained preparations the chromatin forms a thick layer at the membrane and several darkly staining particles in the interior. The nucleus accordingly appears very dark. The large nucleolus is invisible in stained dry smears. The cytoplasm forms a slightly larger accumulation on the indented side of the nucleus. It is homogeneous and basophil; in dry smears it stains pale blue with the Romanowsky eosin-methylene-azure mixture (Fig. 39, *A*, *a*).

In human blood the number of lymphocytes amounts to 20 to 25 per cent of the total number of colorless corpuscles. The relatively scarce larger cells among

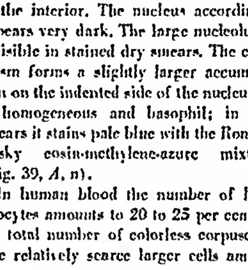

Fig. 41. Lymphocytes of human blood showing cytocentrum. After Weidenreich.

the lymphocytes (Fig. 39, *A: b, c, d*) are looked upon by clinical hematologists as older cells. Their larger size is due to a slightly greater amount of cytoplasm while the nucleus remains unchanged or is less compact.

The cytocentrum is represented by a pair of centrioles located at the indentation of the nucleus (Fig. 41); it is surrounded by a small Golgi net. The mito-

in the normal adult blood varies from 2 to 5 per cent of the total leukocyte count.

The nucleus usually has two oval lobes connected by a thin chromatin thread (Fig. 35, *Eos*). In the young cells, which are rare in the blood of the normal human adult, the connecting bridge is thicker and shorter. In fixed and stained preparations, the lobes of the nucleus show a fairly dense chromatin network, but no nucleoli. A similar nucleus is found in the eosinophil leukocytes of the other mammals. In the rat and mouse it is a thick, irregular ring.

The cytoplasm forms a thin, homogeneous layer at the periphery of the cell. It is more distinct in moving cells where it sends out pseudopodia. In the interior of the cell body the cytoplasm is reduced to thin partitions between the granules and stains faintly with basic dyes. In the middle of the cell body a small area free of granules is occupied by the cytocentrum with its diplosome.

The coarse, highly refringent, specific granules, in man, are spherical and are stained electively with acid dyes; after the usual Romanowsky eosin-methylene-azure stain they are red. (Fig. 39, *II*). Supravital staining with Janus green reveals a few mitochondria between the eosinophil granules. With the exception of some fishes, all vertebrates have typical eosinophil leukocytes in their blood.

The Basophil Leukocytes. These cells are difficult to find in human blood because they form only about one-half of 1 per cent of the total number of leukocytes. Their size is about the same as that of the heterophil leukocytes (see below). In a dry smear they measure 10 μ in diameter. The nucleus is elongated, usually bent in the form of an S, and provided with two or more constrictions. The chromatin network is looser and paler than in the eosinophil leukocytes and does not contain any nucleoli The granules in the cytoplasm of the living cells have a low refractive index. Their substance, in man, is soluble in water and, therefore, in preparations stained with the usual watery dye solutions, the granules are partly dissolved and disfigured. In dry smears or in sections of alcohol-fixed material, the cytoplasm contains round granules of different sizes, which stain a metachromatic purple color with alcoholic thionin or toluidin blue (Fig. 39, *I*). Supravital application of neutral red gives the granules a dark red color.

The solubility of the basophil granules has created considerable confusion in regard to the nature of these cells, but the use of suitable methods leaves no doubt as to their truly specific nature.

The basophil leukocytes show considerable variations in different species of vertebrates. In some mammals, as the guinea pig, their granules are large, oval, insoluble in water, and stain but faintly. In the dog, the granules are very fine and are assembled in a small compact group. In the cat, rat, and mouse, the basophil leukocytes seem to be absent from the blood normally. In the lower vertebrates the variations are still greater.

The Heterophil Leukocytes (Neutrophil in Man). This type of leukocyte is the most numerous and physiologically the most important in the blood of all vertebrates. In fresh human blood these cells can be recognized easily by their fine granulation, which is seen especially well during their ameboid motion (Fig. 35, *Lk*). The size of the spherical cells in fresh condition is from 7 to 9 μ. In a dry smear they measure from 10 to 12 μ, and constitute 65 to 75 per cent of the total number of leukocytes.

The nucleus is highly polymorphous. It is an elongated, bent or twisted body which consists of several irregularly oval or angular lobes connected by very thin chromatin threads. During ameboid motion the nucleus undergoes passive changes of its form, but the constrictions and the thickenings are constant. The number of the lobes varies from 3 to 5 and

creased experimentally in rabbits by infection with *B. monocytogenes* (Murray and co-workers), or by injections of the phospholipins of the tubercle bacillus (Sabin).

Monocytes are often erroneously identified with macrophages, especially in some inflammatory processes where all of the mononuclear exudate cells have been loosely spoken of as constituting a "monocytic reaction" (see p. 109).

The Granular Leukocytes or Granulocytes. In contrast to the lymphocytes and monocytes, the granulocytes always contain granules elaborated in their cytoplasm. These granules are of the same form in any given cell, but are distinctly different in various classes of granulocytes in a given species and in the homologous cells of different species. They may be relatively large or small, spherules or ovoids, or may be irregular in outline, filamentous or rod-shaped. Another general characteristic of the mature granular leukocytes is the shape of their nucleus. Instead of being spherical and slightly indented or kidney-shaped, as in the majority of the nongranular leukocytes, the nucleus of the granulocytes is constricted into a varying number of lobes. The lobated nucleus of the mature cells gradually develops from the compact, spherical nucleus which is found in the young forms (see Fig. 67). In the lower vertebrates the nucleus in the mature granular leukocytes often remains compact. Although different classes of granulocytes may be identified on the basis of their nuclear form and the morphology of the cytoplasmic granules, the most convenient classification is based upon a combination of the morphology and staining reactions of the granules.

In such a classification, the granulocytes fall into three general groups designated as (1) acidophil, (2) basophil and (3) heterophil leukocytes (neutrophil in man). In the first, the granules in the cytoplasm are most often spherical or oval and are electively stained with acid dyes; in the second they are of similar form, but stain electively with basic dyes; while in the third group the granules, although constant in a particular species, differ as to form, size, and staining reaction according to species. (See Fig. 42.)

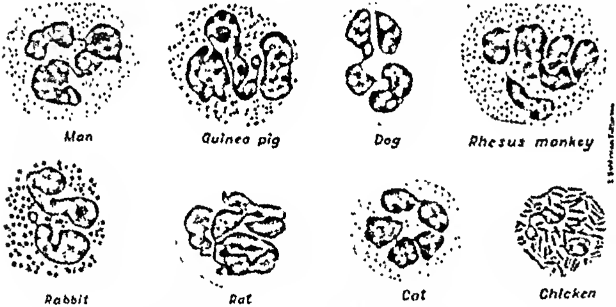

Fig. 42. Heterophil leukocytes of several species. Note the variations in size and staining of the granules in those species in which they are present. Wright's stain.

The Acidophil or Eosinophil Leukocytes. The diameter of these cells, which are spherical in the fresh condition, is about 9 μ. In dry smears the size of the flattened cells is about 12 μ. Their number

phagocytosed inclusions. In animals injected intravenously with vital dyes or corpuscular matter, such as blood cells, large quantities of these substances accumulate in the free macrophages (see p 97). They originate in the spleen, liver, and bone marrow from fixed macrophages through contraction and isolation. They are found especially in the blood of the veins and of the right heart, and the major part of them is filtered off in the capillaries of the lungs, but some may occasionally enter the general circulation.

The free macrophages appear in the blood in certain diseases, especially in those of septic nature. Their presence in the normal blood as claimed by some authors is doubtful and their presence in the blood even in pathologic cases is considered by some to be merely an agonal phenomenon. Confusion has been created in the question of the blood macrophages because many authors did not distinguish these cells from the monocytes. The cells described by some authors in leukemias and other diseases under the name of hemohistioblasts are for the most part the same free macrophages. In some cases, artificially damaged hemocytoblasts or myelocytes in dry smears also seem to have been mistaken for free macrophages or hemohistioblasts.

The Functions of the Leukocytes. Very little is known of the physiologic functions of the leukocytes. For the most part they seem to be inactive cells while in the blood stream. From experiment and pathologic material it is known that they are all able to move, some of them can phagocytose bacteria, others can turn into new cell types, while all of them are very prominent in various types of inflammation.

Amebism. All leukocytes are capable of ameboid motion, although in different degrees. The movements can be observed easily in a drop of fresh blood protected from desiccation and kept at body temperature. The heterophil leukocytes are endowed with the greatest motility of all leukocytes. The factors concerned in the amebism of leukocytes are discussed by DeBruyn.

The clear, marginal layer of cytoplasm or ectoplasm sends out long, pointed pseudopodia; these rapidly become larger and into them flows the endoplasm. The granules stream in various directions, but do not show Brownian movement as long as the cell is alive. When the latter dies and swells Brownian movement usually sets in at once The eosinophil leukocytes are slow in their movements and their lobated pseudopodia have a rounded, smooth edge. The basophil leukocytes move still more slowly. The monocytes are very active, especially in tissue cultures of leukocytes and in the tissues of the living body. If the external conditions are favorable, as in cultures of leukocytes or in the tissues of the living body, even the smallest lymphocytes display a remarkable activity. Their scanty cytoplasm assembles on one side of the nucleus in a rounded mass, which changes its outlines rapidly and sends out small, budlike pseudopodia. While the cell is in progressive motion, the whole cell body and the nucleus are usually drawn out wormlike or are irregularly twisted. The nucleus lies at the anterior end of the moving cell as a rule If movement is on a flat surface, part of the cytoplasm may trail behind.

The amebism of the leukocytes explains why they are not confined to the system of blood or lymph vessels, but may be found everywhere in the connective tissue and occasionally even in other tissues. Under physiologic conditions, single leukocytes, especially lymphocytes, constantly migrate out of the vessels into the tissue, and may at any time return again into the blood or lymph channels. There is a constant exchange of these cells between the blood and the other tissues.

The leukocytes in all probability perform their functions only when outside the vascular system. If these cells are needed in any part of the organism, they assemble rapidly in the blood vessels of the region and migrate into the tissue. This manifests itself on an especially large scale in *inflammation*—the reaction of the tissues to local injuries. The emigration of leukocytes is one of the cardinal phenomena of inflammation, and is easily watched in the living tissue. All stages of this process can also be demonstrated in fixed and stained slides of inflamed tissue (Fig. 89).

Leukocytes by passing through vessel walls may accumulate in enormous numbers in a tissue

is believed to increase with the age of the cell. Under physiologic conditions the majority of the cells have a three-lobed nucleus. In pathologic cases these relations may change considerably. A dark chromatin network is seen in the lobes in stained sections; nucleoli are absent.

The cytoplasm has a peripheral, homogeneous layer which forms the pseudopodia. The inner, slightly acidophil mass is full of very fine granules except for a small, clear area in the center of the cell body which contains the diplosome. In man the granules are stained with *neutral* dyes, and are, therefore, called neutrophil. The Romanowsky mixture gives them a purple hue (Fig. 39, *III*). They can also be stained with acid dyes such as eosin. Supravital staining with Janus green reveals the presence of a few mitochondria. Neutral red applied supravitally gives the granules an indistinct, very pale, yellowish hue. Occasionally, small vacuoles and inclusions of fat or glycogen can be found between the granules.

In other mammals the granules have a variable size and staining reaction which are more or less typical for the species. In the guinea pig and rabbit the granules are stainable with either acid or basic dyes, although they show a predilection for acid dyes and, therefore, were called pseudo-eosinophils. In some species the granules are so small that they are hardly seen with the highest powers of the microscope.

Abnormal Forms of Leukocytes. In some diseases the blood may contain degenerating leukocytes. Vacuoles, droplets of fat or lipoids may appear in the protoplasm. These are well shown by the use of supravital stains. In the granular leukocytes the nucleus may undergo fragmentation into separate parts (*rhexis*) or shrinkage (*pyknosis*). Atypical forms of leukocytes are also known. Such are the *Rieder cells* and the "irritation forms." The first are believed to be senile lymphocytes. They possess the usual basophil protoplasm and a polymorphous, constricted nucleus. The second have a highly basophil, nongranular protoplasm and are considered by some to be plasma cells circulating in the blood. Immature leukocytes enter the blood in certain diseases (see Fig. 39, *C*).

Free Macrophages of the Blood.—Many investigators have described macrophages in the blood. They have a large, eccentric nucleus and a vacuolated, ameboid cytoplasm which often contains

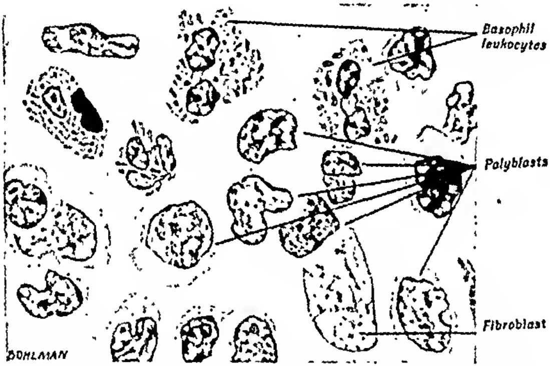

Fig. 43. Basophil granular leukocytes in the local reaction of the connective tissue of a guinea pig to the injection of ventriculin 10 days previously. Hematoxylin-eosin-azure II. 2210 ×. Courtesy of N. Plimpton

phagocytosed inclusions. In animals injected intravenously with vital dyes or corpuscular matter, such as blood cells, large quantities of these substances accumulate in the free macrophages (see p. 97). They originate in the spleen, liver, and bone marrow from fixed macrophages through contraction and isolation. They are found especially in the blood of the veins and of the right heart, and the major part of them is filtered off in the capillaries of the lungs, but some may occasionally enter the general circulation.

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which a short time previously did not contain any. The immigration of the leukocytes, thought to be initiated by positively chemotactic substances which arise in the tissue, reaches its maximum in infections with pyogenic bacteria, where the extravascular tissues become so densely invaded by leukocytes as to obscure the original tissue pattern.

When acute inflammation begins, the colorless corpuscles migrate rapidly into the tissue and infiltrate it. The lymphocytes and monocytes migrate into the tissues together with the heterophil leukocytes, although, at first, usually in smaller numbers. But while the migration of the granulocytes soon subsides, the migration of the agranulocytes extends over a much longer period of time and represents one of the visible manifestations of a more chronic inflammation. As will be shown later, the ultimate fate of these various types of leukocytes in inflammation is quite different (p. 111). The eosinophil and basophil leukocytes also migrate from the blood vessels, but, as a rule, in very much smaller numbers.

Phagocytosis and Other Functions. The heterophil granulocytes display a marked capacity for ingesting small, discrete particles, such as cinnabar, carbon, and bacteria. This action may occur within the circulating blood stream, but is distinctly more extensive in extravascular locations. Such phagocytosis can be watched outside of the body when living leukocytes and bacteria are brought together under suitable conditions.

The phagocytosis of bacteria by the heterophil granulocytes is of great biological importance, as it is one of the means by which the host destroys bacteria, inasmuch as these cells have the power of digesting the included micro-organisms. It is believed that the issue of the infection may sometimes depend directly upon the extent of phagocytosis. In many of the infectious diseases the number of heterophil leukocytes in the circulating blood is markedly increased (*leukocytosis*)

It is easy to show by characteristic reactions that the heterophil granulocytes contain proteolytic and oxidizing enzymes. A positive oxidase reaction is not limited, however, to the granulocytes, but may be displayed also by nongranular leukocytes.

The eosinophil granules give a marked oxidase reaction. The eosinophil leukocytes display phagocytosis but rarely, if ever. Their number increases greatly in the blood when certain animal parasites are in the body. They may accumulate in enormous numbers in local tissue areas, as in the mucous membrane of the respiratory passages in bronchial asthma, or about animal parasites.

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The normal physiologic role of the nongranular leukocytes within the blood stream is as yet undetermined. However, some of their activities, especially in extravascular locations, have been studied extensively. It is known that they bear certain morphologic relations to each other and that they undergo marked transformations, especially in inflammation. Whereas the agranulocytes in the blood stream rarely, if ever, are phagocytic, under suitable extravascular conditions the monocytes, in contrast to the small lymphocytes, are able to engulf particulate matter. The lymphocytes do not give the oxidase reaction; most of the monocytes do. That the lymphocyte plays an important part in the local reaction of a tissue to invasion by malignant tumors is held by a few observers. The lymphocytes accumulate in small numbers about autogenous tissue grafts and in greater numbers about homoplastic tissue grafts. Heteroplastic grafts are surrounded by large numbers of heterophil leukocytes and lymphocytes (Loeb). Dougherty and White believe that lymphocytes are a source of antibodies (page 85).

The Blood Platelets. In the circulating blood the platelets of all mammals are small, colorless corpuscles. They are round or oval, biconvex disks; when seen in profile they look like small, plump spindles or rods. Their size is not quite uniform, the average being 3 μ. Their number varies considerably and is usually given as 250,000 in 1 cu. mm. of blood, although some authors give much higher figures. It is extremely difficult to determine the real number, because as soon as the blood leaves the vessel the platelets adhere to one another and to all surfaces with which they come in contact (Fig. 35).

In a fresh drop of blood the platelets at once agglutinate into small and large clusters and stick to the glass. They are the lightest elements of the blood so that in centrifuged blood they form the uppermost white layer, lose their smooth outlines, and finally disintegrate into small groups of granules. Simultaneously, around and radiating from them, fibrils of fibrin appear in the plasma (Fig. 35).

They may be preserved for observation by rendering the blood incoagulable through the addition of such substances as sodium citrate or heparin. In such blood the platelets remain unchanged for a long time. Of all fixing reagents, solutions of osmic acid preserve them best. In rapidly prepared dry smears, the platelets are preserved as round corpuscles.

After fixation and staining with the Romanowsky mixture, each platelet is seen to consist of two parts. One is highly refractile and contains purple granules. This is the *chromomere*. The other is pale and homogeneous, and stains a pale blue —the *hyalomere*. The chromomere occupies a central or peripheral position. The hyalomere is often seen to send out pointed processes (Fig. 44). Sometimes the platelets contain small vacuoles.

The nature of the platelets is not known. Some older authorities believed them to be unorganized precipitates of the blood plasma. Others considered them to be products of disintegration of erythrocytes, or remnants of the nucleus or the cytocentrum of the red or white blood corpuscles.

At present the dominant opinion connects the origin of the platelets with peculiar giant cells, the megakaryocytes, which are found in the bone marrow of all mammals (p. 92). These cells have a protoplasm which stains a pale blue with the Romanowsky mixture, and contains large numbers of azurophil granules, sometimes arranged in small, dense groups. Some authors claim that pseudopodia-like excrescences, containing azurophil granules, become pinched off the surface of the megakaryocytes and enter the blood stream as platelets. That such is the actual formation of platelets is questioned by recent investigators.

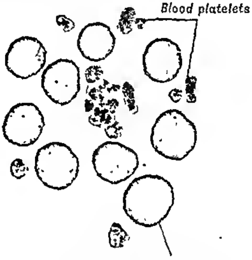

Fig. 44. From a dry, Romanowsky-stained smear of human blood showing platelets with their hyalomere and the dark, punctiform chromomere.

The changes of the platelets in a fresh drop of blood make it very probable that they play a part in the coagulation of the blood. It is believed by some that they are the source of the enzymes which are necessary at least to initiate this process of clotting. However, they cannot be the only source of such enzymes, because blood plasma freed of platelets clots nevertheless. As they are not true cells with a nucleus

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and cytoplasm, the term "thrombocytes" is not applicable to them. Platelets are absent from the blood of the lower vertebrates. These animals have, instead, nucleated spindle cells or thrombocytes, which play a role in blood clotting.

Blood Clotting. Blood, as it circulates normally in the vascular system, is a highly specialized fluid tissue. Upon cessation of the circulation or upon removal of blood from the vessels, however, the fluidity is rapidly lost, the blood becoming a jelly-like mass. This change is termed "blood clotting" or "blood coagulation."

The physical factor involved in this change is the aggregation of a protein of the normal plasma into threads which form an interlacing network, in the small meshes of which are entangled the blood corpuscles and the aqueous menstruum.

The dispersed protein of normal plasma which contributes to the formation of the network is designated *fibrinogen*. This, when modified physically and chemically to form the network, is known as *fibrin*. The liquid fraction of the plasma which remains after the subtraction of fibrin is called *serum* or *blood serum*.

Although many factors are known which modify the speed and extent of fibrin formation, and hence of blood clotting, no ultimate analysis of the phenomenon has been reached. Methods have been developed by which fibrin formation may be so delayed as to allow separation of plasma from the blood corpuscles.

For the details of clotting and the chemical composition of the blood, reference should be made to a text on physiologic chemistry.

The Lymph. The lymph, the liquid which fills the lymphatic vessels, is collected from all over the body and returned to the blood. The composition of the lymph arising in different organs varies markedly. There are no cells in the smallest lymph vessels, the lymph capillaries. As it passes through the lymph nodes, however, more and more cells are added to the lymph. In the thoracic duct it is a more or less opaque, sometimes pinkish liquid which contains large numbers of cells. The lymph here is similar to the plasma of the blood; it also clots, although the clot is much looser and softer. The number of cells in the lymph varies within very wide limits, although their character is uniform. As a rule, besides some few erythrocytes and occasional eosinophil leukocytes, the vast majority, about 99 per cent, are lymphocytes of which the small lymphocytes form 80 to 85 per cent. Medium-sized and, especially, large lymphocytes are relatively rare. Cells of monocytic character occur but very rarely under physiologic conditions; the same is true of larger cells of the macrophage type. Under pathologic or experimental conditions, including tissue cultures of lymph, the cellular aspect of the lymph changes rapidly, and numerous macrophages develop from the lymphocytes.

The lymph from the small intestine contains much fat; during digestion the lymph here has a milky appearance and these lymphatic vessels were therefore called *lacteals*.

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guished in it: (1) the collagenous or white fibers, (2) the elastic or yellow fibers, and (3) the amorphous ground substance.

Collagenous Fibers. All types of connective tissue show collagenous fibers as their most characteristic element. In the loose tissue these are long, straight or wavy threads or ribbons of 1 to 12 μ in thickness. They run in all directions (Fig. 48) and their ends cannot be found. They are colorless and show a more or less distinct, longitudinal striation, while in cross section they seem granular or dotted. Their appearance results from the fact that the fibers consist of extremely fine, parallel, collagenous fibrils, 0.3 to 0.5 μ in thickness, believed to be held together by a cementing substance, presumably a protein, since it is digested by trypsin. On the surface of the fiber the cement substance forms a thin membrane. The fibrils are thought not to branch, but the fibers branch in many places. In electron micrographs the fibrils are cross striated, the distance between the bands is 644 Å on the average. When stained with phosphotungstic acid each striation appears as a series of bands (Fig. 45).

The collagenous fibers are very flexible but offer great resistance to a pulling force; they are not elastic in the common

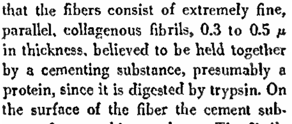


Fig. 45. Electron micrograph of collagen fibril from tendon of rat tail. Phosphotungstic acid stain. 110,000 ×. After Schmitt, Hall and Jakus.

sense of the word. In boiling water, the collagenous substance dissolves and yields a solution of *animal glue* or *gelatin*. In weak acids and alkalis the collagenous fibers swell. Pepsin in acid solution digests the collagenous bundles but they resist alkaline trypsin solution.

A very typical microscopic reaction is obtained with dilute formic acid (Fig. 46). This causes the bundles to swell considerably, lose their longi-

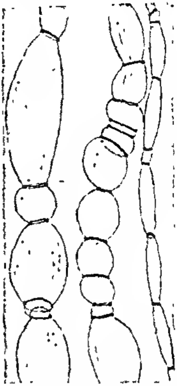


Fig. 46 Swelling of collagenous fibers, from the loose connective tissue of a rabbit, on the addition of ½ per cent formic acid. 185 ×. (A.A.M.)

tudinal striation, and become transparent; in many places these swollen bundles are constricted transversely or obliquely. No satisfactory explanation of this phenomenon has been given as yet. It has been suggested that the cementing membrane contracts and thus cuts deeply into the soft, swelling, collagenous mass, thus forming the constrictions. Another explanation is based on the supposed existence of elastic fibers spirally surrounding the collagenous bundles.

Concentrated acids and alkalis destroy collagenous fibers. Strong acids are therefore used, in the process called *maceration*, to soften the tissues of different organs so that the cells may be separated from the interstitial connective tissue. Collagen gives an insoluble product with salts of heavy metals and with tannic acid; the tanning of leather is based on the treatment of the collagenous feltwork of the skin with tannic acid. Collagenous fibers have no specific staining reactions; however, acid aniline dyes, as for instance the acid fuchsin of the van Gieson stain or the

Chapter IV

THE CONNECTIVE TISSUE PROPER

THE connective tissue proper always contains fibers in its intercellular substance. As this substance and the cells present numerous variations, this type of tissue may be subdivided into numerous categories. The classification is difficult and inexact, for the different categories are linked by transitional forms. Even in the adult organism one type of connective tissue may be directly transformed into another.

The following classification is based on purely morphologic criteria:

- Connective tissue proper
  - A—Loose connective tissue
  - B—Dense connective tissue
  - C—Regular connective tissue
    - Tendinous or ligamentous
    - Fibrous membranes
    - Lamellated
  - D—Connective tissue with special properties
    - Mucous
    - Elastic
    - Reticular
    - Adipose
    - Pigment
    - Connective tissue of the gastro-intestinal and uterine mucous membranes
    - Interstitial connective tissue of lungs, testis, and ovary

THE LOOSE CONNECTIVE TISSUE

The loose connective tissue develops from the mesenchyme which remains after all the other types of the connective tissue have been formed. It contains almost all the cellular and intercellular elements which occur in the other kinds of connective tissue, and serves as a prototype of the connective tissue in general. It is a whitish, sticky mass, which fills out the spaces between the organs and penetrates with the blood vessels into the interior of the organs. When the organs are separated from one another it is stretched between them in thin membranes and threads, and is easily torn during dissection. Like a collapsed sponge it contains innumerable potential cavities which can be easily filled artifically with liquids or air. These are the "cells" of the old anatomists, who are responsible for the name, "areolar tissue," sometimes used.

Intercellular Substance. The intercellular substance forms the main mass of the tissue; three parts can be distin-

cell types: fibroblasts, undifferentiated cells, macrophages, lymphoid wandering cells, mast cells, eosinophils, plasma cells, pigment cells, and fat cells.

Fibroblasts (Fibrocytes, Desmocytes). These are the common connective slender spindles. Their elongated or star-shaped body sends out several spear-shaped processes which end with one or several points. These cell bodies are easily demonstrable with iron hematoxylin but are hard to see in hematoxylin and eosin

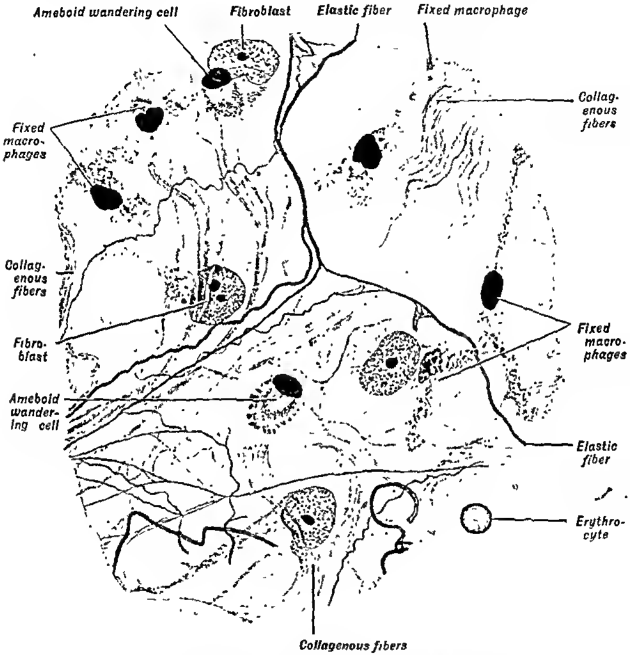


Fig. 48. Section through slightly edematous, subcutaneous, loose connective tissue from the thigh of a man. Iron-hematoxylin stain. 950 ×. (A.A.M.)

tissue cells and are called fibroblasts because they are generally believed to be instrumental in the elaboration of the intercellular fibers. The fibroblasts are long, flat elements which in profile appear as preparations (Fig. 48). The cells are usually adjacent to the surface of the collagenous bundles. The large oval nucleus has a very delicate, sometimes slightly folded, outline and contains dustlike chro-

aniline blue in Mallory's *mixture*, stain the bundles sharply, especially after mordants. Collagen may present physical and chemical differences in various parts of the body and in different species of animals.

Using *x*-ray diffraction methods, Astbury (1940) and others find the collagenous fibers to be composed of parallel bundles of long chains of polypeptides.

Fig. 47 Loose subcutaneous connective tissue from the dorsal surface of a human finger, the connective tissue fibrils are shown in a lamellated ground membrane. 400 X. After Laguesse.

Elastic Fibers. In the loose connective tissue, elastic fibers are scarce. They are very long and run in various directions; they appear as brilliant, highly refractive cylindrical threads or flat ribbons, much thinner than the collagenous fibers. In contrast to the latter, the elastic fibers are not fibrillar, but are usually homogeneous, although the larger fibers may stain more deeply at their periphery; they branch and anastomose freely and form a very loose network (Fig. 48). If the tissue is fixed in its natural position the elastic fibers are straight, while in teased preparations they often appear wavy or spiral. They are highly elastic; that is, they yield easily to stretching but when released at once reassume their former length. When assembled in large numbers they have a yellowish color on macroscopic examination.

The characteristic constituent of the elastic fiber, *elastin*, is also an albuminoid and may vary slightly in its qualities according to its origin. It is highly resistant to boiling water, acids, and alkalis, and through the action of alkalis it can be isolated from the other constituents of the tissue. Elastin is slowly digested by both pepsin and trypsin. Unlike the collagenous fibers, elastic fibers can be stained fairly electively (orcein or resorcin fuchsin). *x*-Ray diffraction studies have yielded inconclusive evidence as to whether elastin should be included in the group of collagen fibers.

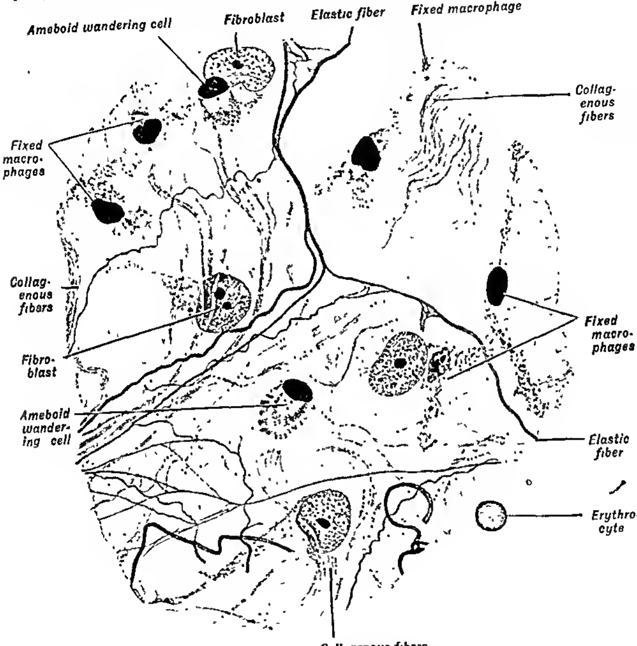
The Amorphous Ground Substance. Most investigators believe that the collagenous and elastic fibers are embedded in a jelly-like, amorphous substance which is related to the cement substance keeping the fibrils together in the fibers. In some places, as in the pulp of the tooth, it may form a continuous mass. However, it is believed to be arranged usually in thin layers, between which remain cleftlike spaces with a minute amount of tissue liquid (Fig. 47). *Hyaluronidase* (spreading factor) liquefies the amorphous substance.

It is very difficult to demonstrate the amorphous substance. In most fresh preparations nothing is seen between the fiber and cells. It is only after a sheet of loose connective tissue is dried on a slide and heavily stained that a thin, pale film between the fibers, can be noticed. As it stains metachromatically with certain dyes (as toluidine blue) it may be one of the complex esters of sulfuric acid which stain in the same way.

The Cellular Elements. The loose connective tissue contains the following

cell types: fibroblasts, undifferentiated cells, macrophages, lymphoid wandering cells, mast cells, eosinophils, plasma cells, pigment cells, and fat cells.

Fibroblasts (Fibrocytes, Desmocytes). These are the common connective tissue cells and are called fibroblasts because they are generally believed to be instrumental in the elaboration of the intercellular fibers. The fibroblasts are long, flat elements which in profile appear as slender spindles. Their elongated or star-shaped body sends out several spear-shaped processes which end with one or several points. These cell bodies are easily demonstrable with iron hematoxylin but are hard to see in hematoxylin and eosin preparations (Fig. 48). The cells are usually adjacent to the surface of the collagenous bundles. The large oval nucleus has a very delicate, sometimes slightly folded, outline and contains dustlike chro-



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The Cellular Elements. The loose connective tissue contains the following

The majority opinion holds that fibroblasts are highly differentiated cells which do not give rise to other types of free cells of the connective substance. There is good evidence that they can develop into osteocytes and some indication that they become phagocytic on intense stimulation. The relation of fibroblasts to mesenchymal and reticular cells has not been determined. (See p. 114.)

Some claim that all the fibroblasts in the tissue are connected with one another through a syncytial fusion of their processes It is more probable, however, that if a connection exists it has merely the nature of a contact. Certainly each fibroblast is an independent cellular element. In inflamed tissue and in tissue cultures it moves independently with a peculiar gliding which does not affect the outer form of the cell body. Ameboid pseudopodia are never observed.

Undifferentiated (Mesenchymal) Cells. Many investigators believe with Marchand that some cells persist in the adult organism with the potencies of undifferentiated mesenchymal cells (Fig. 54). They are often smaller than the fibroblasts but have the same general appearance; in the loose connective tissue they are usually arranged along the blood vessels, particularly along the capillaries (see p. 231). The conviction that they are not common fibroblasts but are undifferentiated cells is gathered from numerous observations which show that under the influence of certain stimuli—as in tissue cultures, inflammation, and the effects of injection of blood toxins—they may undergo progressive development and furnish new cell types. They probably have much the same properties as the *primitive reticular* cells of the blood-forming tissues.

Lymphoid Wandering Cells. These cells present marked variations in size and shape (Fig. 48), and are so irregularly distributed that large stretches of loose connective tissue may be devoid of them. The smallest have a round, darkly staining, very large nucleus and a very scanty, basophil cytoplasm which contains few or no inclusions after supravital staining with neutral red. Such cells resemble the lymphocytes of the blood in every respect.

Many of these wandering cells appear to be monocytes and, among other properties, display a neutral red rosette. The largest cells, which normally are rare, may be 12 μ or more in diameter and have an eccentric, kidney-shaped nucleus and a highly ameboid cytoplasm containing various inclusions when stained supravitally with neutral red. Such elements are structurally identical with the small macrophages (polyblasts) of inflammation (p. 111). A continuous series of gradual transitions can be found between all of these cell types.

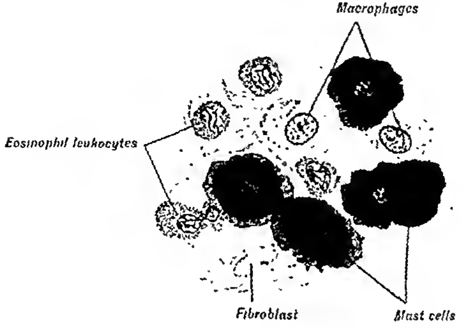
The smaller lymphoid cells are identical with the nongranular leukocytes of the blood. This does not mean that they have all emigrated from the vessels in the adult organism. The cell lineage of many of them originated in the embryonic mesenchyme and stayed there. On the other hand, these cells can always enter the circulation. There is no reason for distinguishing hematogenous and histogenous cells among them; they all have the same potencies.

Fixed Macrophages (Histiocytes). In the loose connective tissue the number of fixed macrophages varies according to the region of the body, the animal species, and so forth. On the average they seem to be almost as numerous as the fibroblasts, which they outnumber in the richly vascular areas. They are scattered singly among the fibroblasts or are assembled in small groups. In inflammation these fixed macrophages become actively ameboid free macrophages (p. 98).

In outer form the fixed macrophages vary from flat, rounded or oval cells to elongated, spindle-shaped elements which sometimes possess branched processes. Ameboid movements are never seen under physiologic conditions. The nucleus is

matin particles and one or more large nucleoli. Near the nucleus are a diplosome and a Golgi net. The mitochondria appear as slender rods which are scarce

The cytoplasm of the resting fibroblast rarely contains inclusions except occasional, small, fat droplets and usually remains colorless when neutral red is ap-



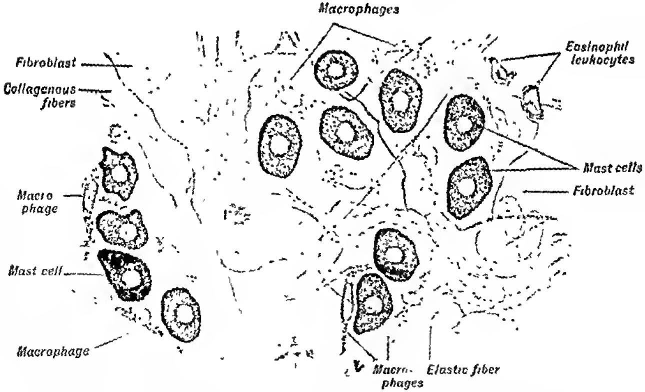


Fig. 49. The two figures are from the loose connective tissue of the rat. Above: Fixed and stained with hematoxylin-eosin-azure 600 ×. Below: Stained supravitally with neutral red. 800 ×. (A.A.M.)

in the processes, but are more numerous near the nucleus and around the cytocentrum. Tonofibrils (also called fibroglia fibers) run along the surface of the cell.

plied supravitally (Fig. 49). In active condition (inflammation and tissue cultures) it contains a number of small neutral red vacuoles.

The homogeneous cytoplasm is strongly basophil and forms a broad layer in the cell body. The middle of the cell is occupied by a round, pale area adjacent to the nucleus; this area is the cytocentrum with its diplosome (Fig. 39, *B*). Scattered around the cytocentrum are mitochondria and a varying number of vacuoles, which stain supravitally with neutral red. These occupy the site of the Golgi net in fixed preparations.

In most of the foci of plasma cells, all transitions from small lymphocytes to fully developed plasma cells can be found, and the process of change has been followed in tissue cultures. The transformation of large lymphocytes (hemocytoblasts) into plasma cells has also been observed.

The plasma cells seem unable to change into any other cell type (except possibly into inflammatory macrophages); they are specifically differentiated elements which finally degenerate. During degeneration, large spherical drops or crystals of a peculiar, acidophil substance frequently accumulate in the cell body. When the cytoplasm disintegrates these inclusions are set free and remain between the other elements of the tissue as *Russell's bodies.*

Eosinophil Cells. In man, these cells are occasionally found in the interstitial tissue of some glands, particularly the mammary gland, and of the lung, and in the omentum. Under certain pathologic conditions they may accumulate in the connective tissue in large numbers. They are numerous in the loose connective tissue of the rat, mouse, and guinea pig (Fig. 49).

Although many investigators believe that cells with eosinophil granules may develop from the local elements of the connective tissue (local eosinophilia), this theory is not sustained by facts. Excluding the connective tissue of the intestinal mucosa where special conditions prevail, the eosinophil cells of the connective tissue are eosinophil leukocytes which have migrated from the blood vessels and have settled in the tissue.

The nucleus in these cells always has the typical, polymorphous character of the matnre eosinophil leukocyte of the respective species. Thus, in the rat or mouse it is a thick ring. Round, compact nuclei, as well as mitoses, which are typical for the young forms (myelocytes) of the eosinophil leukocytes, do not occur in the connective tissue.

Pigment Cells. In the loose connective tissne of the mammals, pigment cells are very rare; they occur more frequently in the dense connective tissue of the skin. They are small, elongated cells with short irregular outgrowths; the cytoplasm contains small granules of *melanin.* The pigmented cells in the superficial layers of the derma are believed by many to be merely connective tissue phagocytes which receive their pigment from the epithelium; they may be called *dermal chromatophores.* Pigment cells containing melanin which they themselves have elaborated occur in the derma in the sacral region of the newborn of the Mongolian race. These cells had best be called *dermal melanoblasts,* to distinguish them from the *epidermal melanoblasts.* (See section on the skin.) Melanoblasts are found in most mammals in the pia mater of the ventral surface of the medulla oblongata. A large number of melanoblasts is assembled in the pigment tissue of the chorioid of the eye, where they are flattened and have broad, lobated processes (Chapter XXX).

The pigment granules in the melanophores in hypophysectomized frogs retract about the nucleus. After the injection of extracts of the pars intermedia of the hypophysis and exposure of the frogs to sunlight, the pigment granules extend rapidly throughout the processes of these cells.

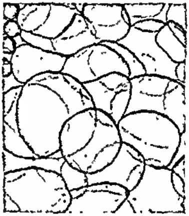


Fig. 50. Fresh preparation of a human fat lobule, showing fat cells and smaller, extracellular fat drops (more highly refractile). 160 ×. After Schaffer.

The pigment cells arise early in ontogenetic development from neural crest cells in amphibians (DuShane) and birds. Their origin in mammals is not clear.

Fat Cells. Small droplets of neutral fat may occur in any cell of the connective tissue. There are, however, cells with a special fat-storing function; only these should be termed "fat cells." They are often found scattered singly or in groups in the loose connective tissue, especially along the blood vessels. When they accu-

smaller than that of the fibroblast, has a heavy, slightly folded membrane, and is irregular, oval or kidney-shaped in form. It contains no large nucleoli; the chromatin particles are coarser and stain darker than in the fibroblasts. The cytoplasm has distinct, ragged outlines and stains darkly (Fig. 48). Near the nucleus there is a distinct diplosome and a Golgi net; the mitochondria are short rods or granules and are assembled mainly around the cytocentrum. The cytoplasm usually contains a number of small vacuoles which stain supravitally with neutral red (Fig. 49). These cells are able to ingest many types of particulate matter—see p. 97.

A characteristic property of the fixed macrophages is their elective storing of certain electronegative, acid aniline dyes in colloidal solution, such as trypan blue and lithium carmine. After injection of a solution of one of these dyes into the living animal, the fixed macrophages accumulate granules of the dyestuff in their cytoplasm (Fig. 8, 5). If repeated injections are made, the dye inclusions become very numerous and large, and the tissue develops a distinct, macroscopic color. As the fibroblasts store little or none of the dye, this method allows a precise identification of these macrophages.

The degree of the dye storage depends largely upon the degree of the dispersion and amount of the solution used and upon the mode of introduction into the body. The most intense storage is obtained where the concentrated dye solution has immediate access to the cells, as in a subcutaneous injection The vital storage of acid dyes has been looked upon as a phagocytosis of ultramicroscopic particles.

In the loose connective tissue of the mammals, transitional forms between the fixed macrophages and the fibroblasts are very rare under physiologic conditions, as are transitions between the lymphoid wandering cells and the macrophages. But in inflamed tissue the sharp limits between the cells are effaced in certain stages (p. 111). The distinction between these different cell types may also be difficult in the lower vertebrates.

The fixed macrophages of the loose connective tissue have also been called *clasmatocytes, rhagiocrine cells, adventitial cells, histiocytes, resting wandering cells*, and many other names.

Mast Cells. Mast cells have been found, often in groups about the blood vessels, in the connective tissue of most vertebrates. Their cytoplasm is filled with granules which stain metachromatically with basic aniline dyes; with methylene blue or thionin the granules have a purple color. Neutral red stains them supravitally a dark, brick red (Fig. 49). The granules in some species are soluble in water.

In the rat and mouse, the mast cells are very large and spherical or polyhedral; in other mammals, including man, they are smaller, irregularly oval or flattened cells. Very slow amebism can be observed occasionally. The spherical, relatively small nucleus is quite inconspicuous. In the neighborhood of the nucleus is a diplosome.

In mammals the mast cells of the connective tissue and the basophil leukocytes of the blood are independent cell types despite the similar properties of the granules. The function of the mast cells is not known, although Holmgren, Jorpes and Wilander believe their granules consist of heparin. They base this conclusion on similarity of staining reactions and on experiments which show a parallelism between the extractible heparin and the mast cell content of certain organs.

Plasma Cells. In the common connective tissues, plasma cells are extremely rare, although they occur quite frequently in the serous membranes and in the lymphatic tissue. They may be only as large as a small lymphocyte, or may be two or three times that size. Their form is spherical and often flattened. In the living cell the spherical or polygonal body has a glassy, homogeneous appearance. Very slow movements can sometimes be observed.

The nucleus is small, round or slightly oval, and has an eccentric position. In its interior and at the membrane, coarse, darkly staining, regularly distributed chromatin particles are seen. Mitoses are exceedingly rare.

cultures of adult loose connective tissue, the new formation of fat cells from the fibroblast-like cells has been observed very often. In ear chambers in living rabbits, the development of fat cells from fibroblast-like cells has been observed.

The fibroblastic nature of the cells which are transformed into fat cells has not been proved conclusively. It is possible that when new fat cells develop in the adult they arise from the undifferentiated mesenchymal cells. This would agree with the fact that new fat cells—as in the embryo—always appear along the small blood vessels, which are accompanied by cells of undifferentiated mesenchymal nature.

The first step in the formation of a fat cell is the appearance of a few small droplets of fat in the cytoplasm (Fig. 51). These increase in size and gradually fuse. Simultaneously, the cell body swells, the processes are withdrawn, and the cell becomes spherical. As the fat drop increases in size, the cytoplasmic layer is reduced to a thin membrane while the nucleus is compressed and flattened. Mitoses are not found in fat cells. When the fat cell loses its fat, the same series of transitional forms can be observed in the reverse sequence. When the fat droplets become very small, the cytoplasm sends out tapering processes and gradually the cell regains the appearance of a fibroblast. The question of the exact nature of the cells into which the fat cells are transformed when they lose their fat is unsettled. They by no means always resemble true fibroblasts, but may remain, as in serous atrophy, in the tissue as peculiar, sharply outlined, spherical or oval elements (Fig. 52). This has been confirmed by studies on living cells in chambers in the rabbit's ear (Clark and Clark).

The fat enters and leaves the cell in the invisible form of its soluble components. The idea that very small droplets of fat (hemoconia) may penetrate the membrane and fuse with the large fat drop has not been confirmed.

The Functions of the Loose Connective Tissue. The loose connective tissue is the medium supporting and surrounding the elements of the other tissues. It serves as packing material and fills out the spaces between the organs, and its flexible collagenous fibers allow a more or less distinct movement of the connected parts in relation to one another. This is its mechanical function.

In addition, this tissue plays an important role in the nutrition of the elements of other tissues which are embedded in it. The part each cell type takes in this function is unknown. But it is clear that all substances which the cells of the other tissues receive from the blood, and all the products of metabolism and water which they turn over to the blood and lymph

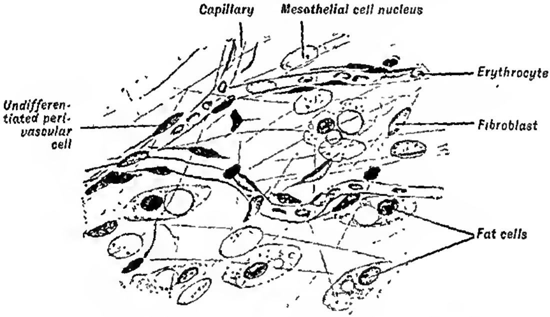


Fig. 52. Portion of the omentum of a starving rabbit. The fat cells have lost most of their fat and appear with much cytoplasm. 375 ×. (A.A.M.)

mulate in large numbers and crowd out the other cells the tissue is transformed into fat or *adipose tissue* (p. 75).

A living fat cell is a large, brilliant, spherical body (Fig. 50). The nucleus can be seen only if it occupies a lateral position. If the cell is damaged, liquid neutral fat runs out in drops, although in

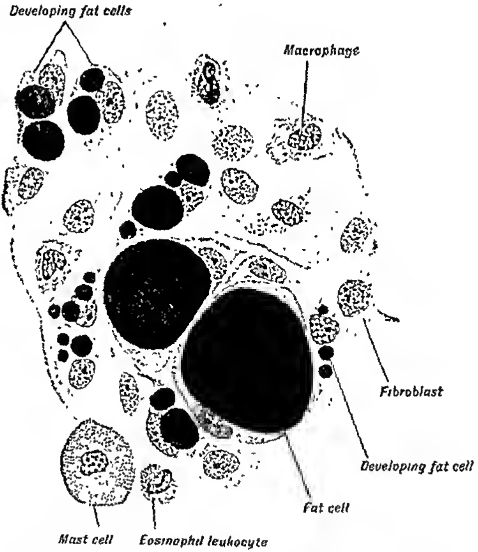


Fig. 51. Several fat cells from the subcutaneous, loose connective tissue of a rat. The fat has been stained black by the osmic acid of the fixation fluid. About 1000 ×. (A.A.M.)

many warm-blooded animals the fat becomes solid after cooling. Every mature fat cell contains only one large drop of neutral fat, which can be stained black with osmic acid (Fig. 51) or orange with Sudan III. The cytoplasm is reduced to a thin membrane which surrounds the drop; it is thickened in that part which contains the flattened nucleus with its heavily staining, central mass of chromatin.

In the brown fat tissue (interscapular gland) the cells, as a rule, contain not one large but several small fat droplets which are separated by a relatively abundant cytoplasm (Fig. 64). After the fat is dissolved such cells have a coarse, alveolar structure. Similar conditions are often observed in the fat cells of the lower vertebrates.

New fat cells *may* develop at any time in the connective tissue of the adult organism and fully developed fat cells may lose their fat when the organism does not receive sufficient nutritive material. It is generally believed that any fibroblast may become transformed into a fat cell. In tissue

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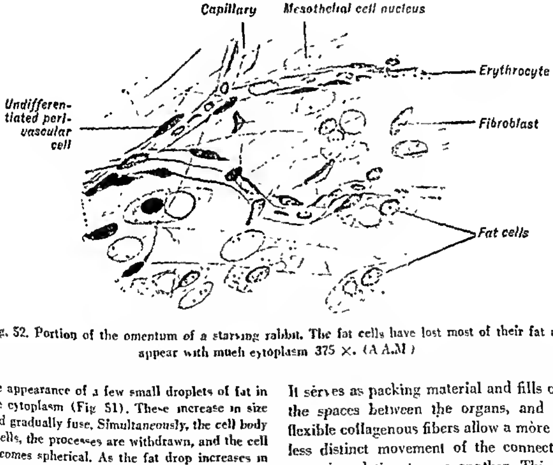

Fig. 52. Portion of the omentum of a starving rabbit. The fat cells have lost most of their fat and appear with much cytoplasm 375 ×. (A.A.M.)

In addition, this tissue plays an important role in the nutrition of the elements of other tissues which are embedded in it. The part each cell type takes in this function is unknown. But it is clear that all substances which the cells of the other tissues receive from the blood, and all the products of metabolism and water which they turn over to the blood and lymph

mulate in large numbers and crowd out the other cells the tissue is transformed into fat or *adipose tissue* (p. 75).

A living fat cell is a large, brilliant, spherical body (Fig. 50). The nucleus can be seen only if it occupies a lateral position. If the cell is damaged, liquid neutral fat runs out in drops, although in many warm-blooded animals the fat becomes solid after cooling. Every mature fat cell contains only one large drop of neutral fat, which can be stained black with osmic acid (Fig. 51) or orange with Sudan III. The cytoplasm is reduced to a thin membrane which surrounds the drop; it is thickened in that part which contains the flattened nucleus with its heavily staining, central mass of chromatin.

In the brown fat tissue (interscapular gland) the cells, as a rule, contain not one large but several small fat droplets which are separated by a relatively abundant cytoplasm (Fig. 64). After the fat is dissolved such cells have a coarse, alveolar structure. Similar conditions are often observed in the fat cells of the lower vertebrates.

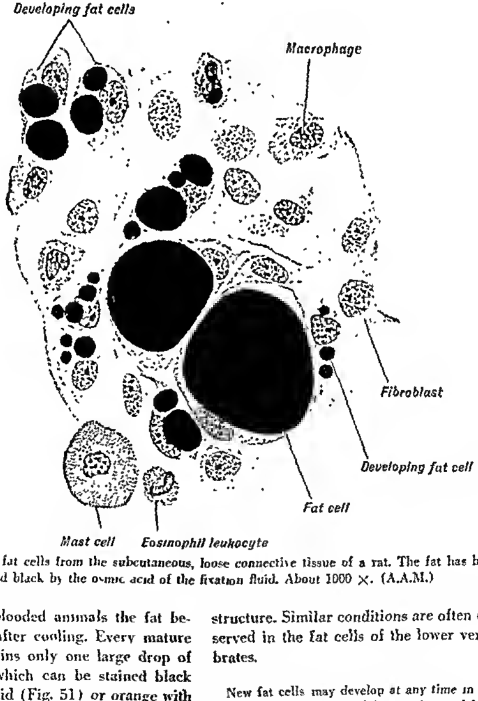

Fig. 51. Several fat cells from the subcutaneous, loose connective tissue of a rat. The fat has been stained black by the osmic acid of the fixation fluid. About 1000 ×. (A.A.M.)

New fat cells may develop at any time in the connective tissue of the adult organism and fully developed fat cells may lose their fat when the

erties make up the so-called "constitution" and are probably intimately connected with the properties of the diffuse connective tissue. The altered conditions of the allergic or hypersensitive organism (an organism which under the influence of a given stimulus has changed its reac-

tissue. On the other hand, if a graft of a carcinoma "takes" and grows, the connective tissue furnishes the nutritive stroma for the malignant epithelial cells and both tissues are combined into a new unit.

The Tissue of the Serous Membranes. The serous membranes (the

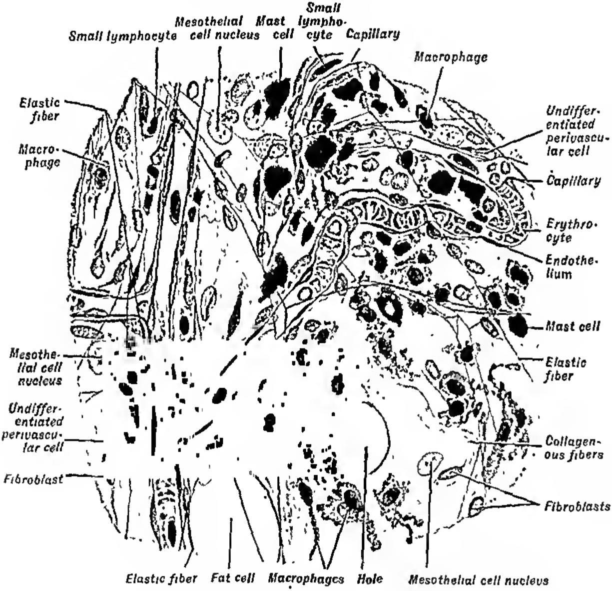

Fig. 54. Stretch preparation of the omentum of a man. Hematoxylin-eosin-azure stain. 450 ×. (A.A.M.)

tion to this stimulus) are sometimes manifested by alterations of the loose connective tissue.

The development of a tumor is influenced to a high degree by the behavior of the loose connective tissue. The failure of a tumor to "take" when inoculated into an animal is probably the result of a successful reaction of the connective

peritoneum, the *pleura*, and the *pericardium*) are thin layers of loose connective tissue covered on their free surfaces by a layer of mesothelium. When the membranes are folded, as the omentum or the mesentery, both of the free surfaces are covered with mesothelium. The serous cavities always contain a small amount

must pass through a layer of connective tissue. It has been claimed that some of the cells of this tissue have an endocrine function, but the evidence supporting this view is not convincing.

Of great importance is the role the loose connective tissue plays as the arena of the local reactive process called *inflam-* local reaction which manifests itself in a series of complicated phenomena in which local elements of the connective tissue as well as blood cells participate. The results of this local reaction are the destruction, digestion, and absorption of the foreign noxious substances and the reparation of the damage caused by them.

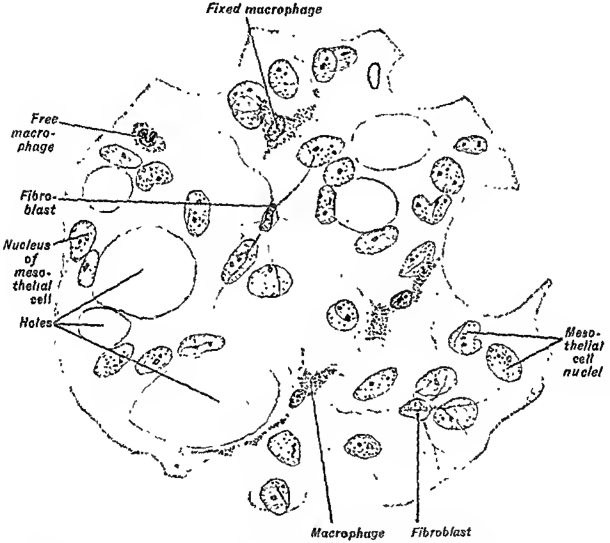

Fig. 53. Stretch preparation of a thin part of the omentum of a rabbit injected with lithium carmine. 375 ×. (A.A.M.)

mation. Even under physiologic conditions, endogenous noxious substances may appear in various places in the body. Some of them have to be neutralized or destroyed by the elements of the connective tissue; this process has been termed "physiologic inflammation." Exogenous stimuli, which cause a pathologic inflammation, call forth a much more intense

Generalized noxious stimuli are taken care of by the diffuse connective tissue and the blood-forming tissues. The main role in this case is played by the macrophages.

The development and issue of local and generalized inflammation and infections are markedly influenced by the individual peculiarities of the organism. These prop-

erties make up the so-called "constitution" and are probably intimately connected with the properties of the diffuse connective tissue. The altered conditions of the allergic or hypersensitive organism (an organism which under the influence of a given stimulus has changed its reaction to this stimulus) are sometimes manifested by alterations of the loose connective tissue.

The development of a tumor is influenced to a high degree by the behavior of the loose connective tissue. The failure of a tumor to "take" when inoculated into an animal is probably the result of a successful reaction of the connective tissue. On the other hand, if a graft of a carcinoma "takes" and grows, the connective tissue furnishes the nutritive stroma for the malignant epithelial cells and both tissues are combined into a new unit.

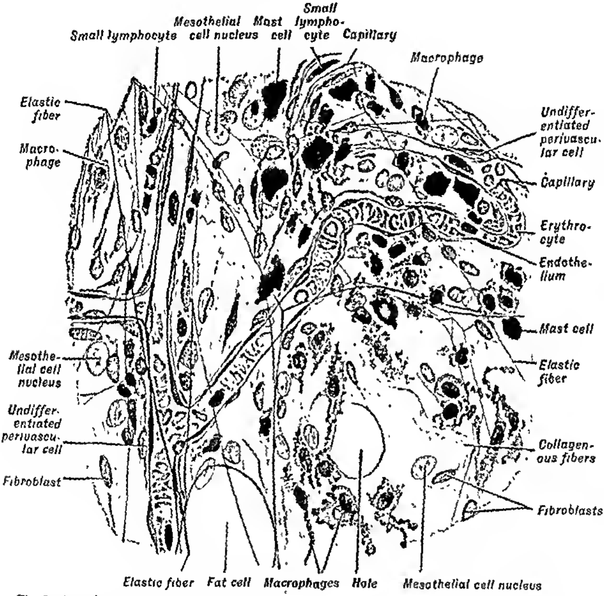

Fig. 54. Stretch preparation of the omentum of a man. Hematoxylin-eosin-azure stain. 450 ×. (A.A.M.)

The Tissue of the Serous Membranes. The serous membranes (the *peritoneum*, the *pleura*, and the *pericardium*) are thin layers of loose connective tissue covered on their free surfaces by a layer of mesothelium. When the membranes are folded, as the omentum or the mesentery, both of the free surfaces are covered with mesothelium. The serous cavities always contain a small amount

of serous liquid, the *serous exudate.* The cells floating in it originate from the serous membrane.

All the elements of the loose connective tissue are found in the serous membranes, where they are arranged in a thin layer. The mesentery contains a loose network of collagenous and elastic fibers, scattered fibroblasts, fixed macrophages, mast cells, and a varying number of fat cells along the blood vessels.

Physiologically the most important, and histologically the most interesting part of the serous membranes in mammals is the *omentum.* It is composed, not of lymphatic tissue as some recent authors hold, but of loose connective tissue. Parts of this membrane contain vessels accompanied by fat tissue. Other parts have either no vessels or only a very few small ones; here the membrane is pierced by innumerable holes and is thus reduced to a fine lacelike net (Fig. 53). The threads of the net are formed by collagenous bundles; between them there are usually no cells although the threads are covered by flat mesothelial cells. In such places the collagenous fibers must have been produced by the mesothelial cells.

In those areas of the omentum which are not provided with holes, the tissue contains the same cell types as the common loose connective tissue, but in the omentum the cellular elements are much more numerous and active. Undifferentiated mesenchymal cells are found along the vessels. Especially numerous are the fixed macrophages which here are highly elongated and often branched in form (Fig. 54). Many of them are mobilized into free macrophages, which contain dye inclusions in a vitally stained animal. There are very many small lymphocytes and plasma cells and, occasionally, eosinophil leukocytes and mast cells. The number of lymphocytes and plasma cells varies considerably in different animals

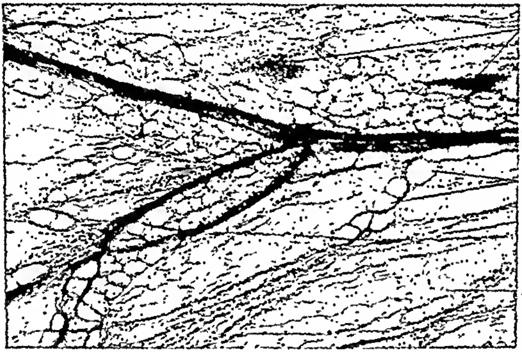

Fig. 55. Photomicrograph of stretch preparation of omentum of Macacus rhesus. Hematoxylin-eosin-azure II. 55 ×.

The great individual variations in the structure of the omentum are explained by the rôle it probably plays in the absorption and neutralization of various endogenous noxious products of metabolism ("physiologic inflammation"). When exogenous noxious factors penetrate the peritoneum, this activity of the omentum is greatly

intensified, and the number of cells increases still more. In such cases heterophil granular leukocytes migrate from the vessels into the omentum.

In certain areas the fixed and free macrophages are accumulated in dense masses Such areas are often arranged along the blood vessels as small or large, round or oval patches, called *milky spots.* These may or may not contain blood vessels, and are sometimes also found in the netlike part of the omentum. They are very characteristic in the omentum of the rabbit.

In the milky spots, the fixed macrophages proliferate through mitosis, round off, and migrate the milky spots of the omentum and migrate into the cavity. They correspond with those polyblasts of the inflammatory exudate which originate through mobilization of the local fixed macrophages (p. 114).

2. Desquamated mesothelial cells (Fig. 56, *Mes*). These keep their squamous form or they become spherical with small, budlike protuberances. The nucleus usually contains a heavily staining nucleolus. In inflammation and tissue culture these cells develop into fibroblasts.

3. Small lymphocytes (Fig. 56, *Sl*). The vast majority of these have migrated from the blood

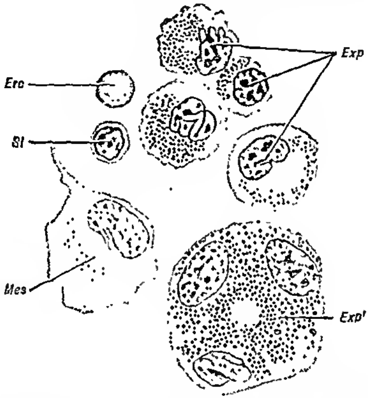

Fig. 56. Cells from the peritoneal exudate of a rabbit injected intravenously with lithium carmine: *Mes,* Desquamated mesothelial cell; *Sl,* small lymphocyte; *Exp,* carmine-storing exudate polyblasts; *Exp',* exudate polyblast with three nuclei; *Erc,* erythrocyte. Moist fixed smear. Note carmine granules (dark gray). Hematoxylin stain. 1000 ×. (A.A.M.)

through the covering layer of mesothelium into the peritoneal cavity. In the serous membrane which lines the pleural cavities there are also cellular areas much like the milky spots of the omentum.

The Free Cells of the Serous Exudate. Normally the amount of serous exudate is very small but in pathologic conditions it may increase enormously. The exudate contains a number of freely floating cells. Among them the following can be distinguished:

1. Spherical ameboid cells which store vital dyes and phagocytose particulate matter (Fig. 56, *Exp*). These are macrophages which originate in vessels of the omentum. Some few may have developed through proliferation of the undifferentiated mesenchymal cells of the omentum In inflammatory exudates, transitions from the lymphocytes to the large macrophages can be found in great numbers.

4. In some animals (guinea pig) eosinophil leukocytes of hematogenous origin occur.

5. In the rat and mouse there are free connective tissue mast cells

6. In pathologic inflammatory exudates there are great numbers of heterophil leukocytes from the blood.

The Leptomeninges. The tissue of the pia

mater and arachnoid is also a variety of the loose connective tissue. It is described in the section on the Nervous Tissue (see p. 223).

THE DENSE CONNECTIVE TISSUE

This tissue is found mainly in the derma of the skin and the submucous layer of the intestinal and parts of the urinary tracts. Its constitution in the derma is typical. The elements are the same as in the loose variety, but the collagenous bundles are thicker and are woven into a very compact feltwork. They are accompanied by many elastic networks. All of the fibers from the derma continue directly into those of the loose, subcutaneous tissue where their arrangement is correspondingly looser. Between the two kinds of fibers and the amorphous cement substance which keeps them together are small clefts which contain the cells; these are much more difficult to identify than in the loose tissue where they can expand in the large spaces. The fixed macrophages are easily recognized in vitally stained animals. Along the small vessels there are always many inconspicuous nuclei which probably belong to undifferentiated mesenchymal cells.

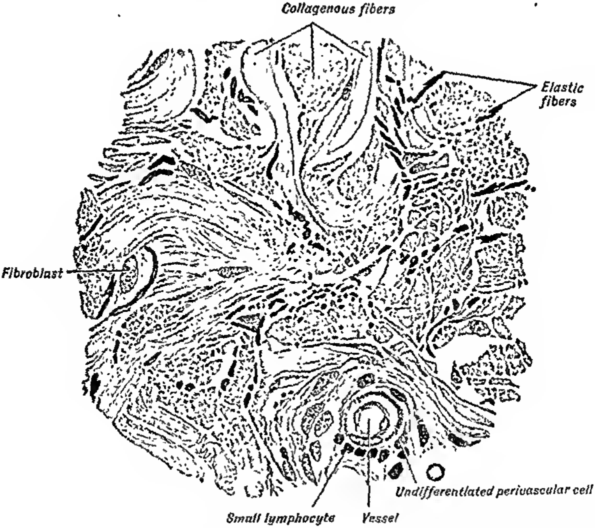

Fig. 57. Section of the derma of a man: dense, irregularly arranged connective tissue. Orcein and hematoxylin stains. 500 ×. (A.A.M.)

THE REGULAR CONNECTIVE TISSUE

The constituents of the regular connective tissue, especially the collagenous bundles, are arranged according to a definite plan. The particular arrangement reflects the mechanical requirements of the particular tissue, whether a tendon, a fibrillated membrane, or lamellated connective tissue.

Tendons. Here the fibers form a flex-

ible tissue which offers great resistance to a pulling force. Macroscopically the tissue has a distinct fibrous structure and a characteristic, shining, white appearance.

The chief constituents of the tendon are thick, closely packed, parallel, collagenous bundles (Figs. 58, 59), in structure the same as those in the loose connective tissue. They show a distinct longitudinal striation and in many places fuse with one another at very acute angles. In cross section they appear as finely dotted areas, usually separated from one another by broken, angular lines, although often continuing into one another. Very fine elastic networks have been described between the collagenous bundles.

The fibroblasts are the only cells present; they are arranged in long, parallel rows in the spaces between the parallel collagenous bundles. The cell bodies are rectangular, triangular, or trapezoid, when seen from their surface, and rod-shaped when seen in profile. Their cytoplasm stains darkly with basic dyes and contains a clear attraction sphere adjacent to the single, round nucleus.

Although the limits between the successive cells in a row are distinct, the lateral limits of the cells are indistinct because here the cytoplasm continues into a very thin membrane. Sometimes it can be followed in the transverse direction to an-

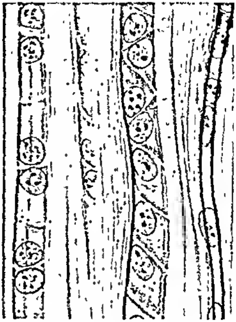

Fig. 58. Freshly teased tendon of the tail of a rat, stained with methylene blue. The rows of tendon cells run between the collagenous bundles. 380 ×. (A.A.M.)

other cell row. In a stained cross section of a tendon, the cells appear as dark, star-shaped figures between the collagenous bundles (Fig. 59). A tendon consists of a varying number of small tendon bundles which are bound by loose connective tis-

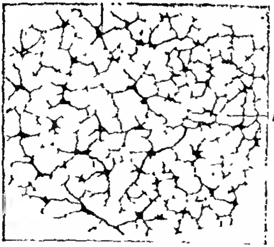

Fig. 59. Cross section of a tendon from the tail of a rat. Hematoxylin-eosin-azure II stain. About 350 ×. (A.A.M.)

mater and arachnoid is also a variety of the loose connective tissue. It is described in the section on the Nervous Tissue (see p. 223).

THE DENSE CONNECTIVE TISSUE

This tissue is found mainly in the derma of the skin and the submucous layer of the intestinal and parts of the urinary tracts. Its constitution in the derma is typical. The elements are the same as in the loose variety, but the collagenous bundles are thicker and are woven into a very compact feltwork. They *are accompanied by many elastic networks*. All of the fibers from the derma continue directly into those of the loose, subcutaneous tissue where their arrangement is correspondingly looser. Between the two kinds of fibers and the amorphous cement substance which keeps them together are small clefts which contain the cells; these are much more difficult to identify than in the loose tissue where they can expand in the large spaces. The fixed macrophages are easily recognized in vitally stained animals. Along the small vessels there are always many inconspicuous nuclei which probably belong to undifferentiated mesenchymal cells.

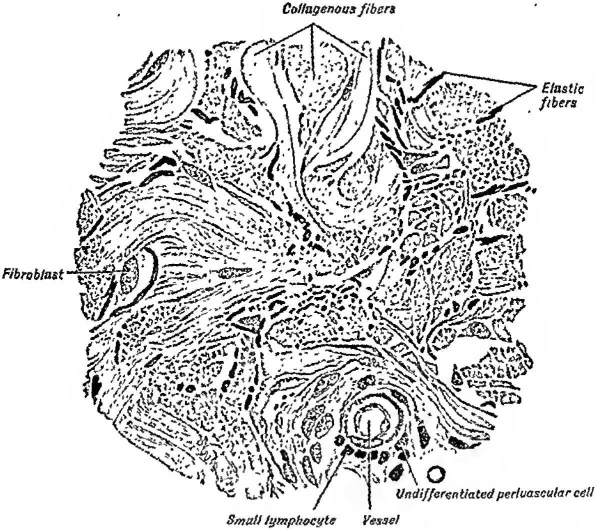

Fig. 57. Section of the derma of a man: dense, irregularly arranged connective tissue. Orcein and hematoxylin stains. 500 ×. (A.A.M.)

THE REGULAR CONNECTIVE TISSUE

The constituents of the regular connective tissue, especially the collagenous bundles, *are arranged according to a definite plan*. The particular arrangement reflects the mechanical requirements of the particular tissue, whether a tendon, a fibrillated membrane, or lamellated connective tissue.

Tendons. Here the fibers form a flex-

macrophages and lymphoid wandering cells are also present. The intercellular substance is soft, jelly-like and homogeneous in fresh condition; when fixed it contains granules and fibrillar precipitates. It gives the reaction for mucin and contains thin, collagenous fibers which increase in number with the age of the fetus.

In the walls of large arteries (p. 238), the spaces between the elastic membranes and the cells are filled with a mucoid intercellular substance, which stains metachromatically with blue, basic aniline dyes. The basophilia of this ground substance is believed to be due to the presence of chondroitin sulfuric acid.

The peculiar mucoid connective tissue of the comb of the capon increases greatly when this animal is injected with male hormone; this reaction has been extensively used in the assay of this material.

Elastic Tissue. Some parts of the body have an elastic tissue which yields easily to a pulling force but regains its original length as soon as the tension is released. Here the elastic fibers predominate and the tissue has a yellow color macroscopically. It may appear in the form of strands of parallel fibers, as in the ligamenta flava of the vertebrae, in the true vocal cords, in the ligamentum stylohyoideum, the ligamentum suspensorium penis, in the tendons of the smooth muscle of the trachea. In these situations the elastic fibers are thick, refringent, and round or flattened; they branch frequently and fuse with one another at acute angles, as in a stretched fishing net. In cross sections, the angular or round areas representing the fibers form small groups; the spaces between the elastic fibers are filled with a delicate feltwork of collagenous fibers and a few fibroblasts.

The elastic tissue forms membranes in the walls of hollow organs upon which a changing pressure acts from within, as in the largest arteries, in some parts of the heart, in the trachea and bronchi.

In the large arteries the structural unit of the elastic tissue is a *fenestrated membrane,* a lamella of *elastin* of variable thickness provided with many irregular openings (Fig. 214). The fenestrated membranes are arranged in many layers around the cavity of the organ and are connected with one another by oblique, ribbon-like branches. The spaces between the lamellae contain a mucoid amorphous mass and smooth muscular cells with irregular outlines. It is impossible to distinguish sharply between the fibrous elastic networks and the fenestrated elastic membranes.

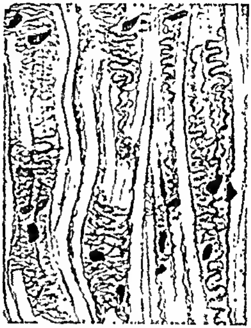

Fig. 61. Longitudinal section of a part of the ligamentum nuchae of an ox. The thick, bright, gleaming elastic fibers form most of the tissue. Between them are dark fibroblast nuclei and wavy collagenous fibers. Eosin-azure stain. 300 ×. (A.A.M.)

Reticular Tissue; Argyrophil Fibers. Although the collagenous fibrils are generally believed not to branch, in certain places there are systems of nonelastic connective tissue fibers which form networks. This fact was established first for the reticular framework of the lymphatic and myeloid tissues and for the red pulp of the spleen. Later, similar, but more

sue into larger bundles (Fig. 60). (See also Fig. 98).

The *ligaments* are similar to the tendons, except that the elements are less regularly arranged.

Fibrous Membranes. The tissues of this group form membranes which surround various organs. On examination with the naked eye they show, in the fasciae, aponeuroses, and tendinous center of the diaphragm, a parallel fibrillation and are white and shiny like the tendons. In other situations the fibrillation is less regular and the

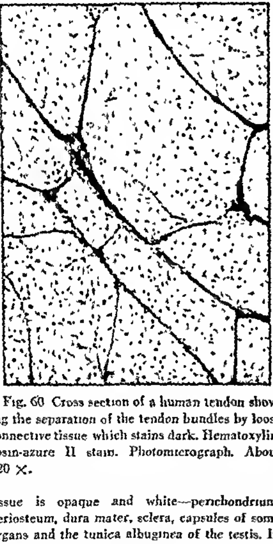

Fig. 60 Cross section of a human tendon showing the separation of the tendon bundles by loose connective tissue which stains dark. Hematoxylin-eosin-azure II stain. Photomicrograph. About 120 ×.

tissue is opaque and white—perichondrium, periosteum, dura mater, sclera, capsules of some organs and the tunica albuginea of the testis. In the cornea of the eye the tissue is transparent.

In the fasciae and aponeuroses the collagenous bundles and fibroblasts are arranged regularly in sheets. In each sheet the fibers follow a parallel and often slightly wavy course In the different sheets the direction may be the same or it may vary; the fibers often pass from one sheet into another and, therefore, a clear isolation of the sheets is seldom possible. Between the collagenous bundles, fine networks of elastic fibers are usual. The cells correspond to the tendon cells and adapt themselves to the spaces between the collagenous bundles.

In the fibrous membranes with somewhat less regularly arranged elements (periosteum, sclera, etc.), a section perpendicular to the surface shows layers of collagenous bundles cut in the longitudinal, oblique, or transverse directions, and cells which are irregular, flat, or fusiform. In these tissues there are always gradual transitions to places where the elements have a quite irregular, dense arrangement. There is also no sharp distinction between them and the surrounding loose connective tissue.

Lamellated Connective Tissue. The lamellated connective tissue is found where small organs or parts of organs, usually of cylindrical shape, need thin and soft but resistant, protective sheaths. It may be looked upon as a condensation of the loose connective tissue on the surface of these cylindrical structures. The elements cannot be sharply separated from those of the loose connective tissue.

Lamellated connective tissue is found outside the basement membrane in the wall of the seminiferous tubules in the testis; in the perineurium, which ensheaths the bundles of nerve fibers in a nerve trunk; and in the outer capsule of some sensory nerve endings, especially the corpuscles of Pacini (Fig. 182).

In a cross section through any of these structures, the periphery is found to be surrounded by a number of concentric, sometimes dotted lines between which are thin, rod-shaped nuclei. The lines are cross sections of thin lamellae, parallel or irregularly arranged collagenous fibers in an amorphous cement substance. These lamellae also contain elastic networks and reticular fibers (p. 73). The surface of the lamellae is covered with a layer of flattened, endothelium-like fibroblasts, whose outlines can be made distinct by the use of silver nitrate. Fixed macrophages are also present.

CONNECTIVE TISSUE WITH SPECIAL PROPERTIES

Mucous Connective Tissue. This tissue is found in many parts of the embryo, as under the skin, and is a form of the loose connective tissue. The classical object for its study is *Wharton's jelly of the umbilical cord* of the human fetus. The cells are large, stellate fibroblasts whose processes often seem to fuse with those of neighboring cells. A few fixed

collagenous tissue is formed in the embryo or in the adult body are argyrophil networks; these are gradually transformed into collagenous bundles. The idea of the immature nature of the reticular fibers agrees well with the fact that they are usually found in those places in the connective tissue where undifferentiated cells of mesenchymal nature are assembled (lymphatic and myeloid tissues and outside the walls of the capillaries).

Adipose Tissue. According to most authors fat tissue is loose connective tissue in which fat cells have displaced most of the other elements. Thus, no sharp limit can be drawn between the two tissues. The fat cells are closely packed and form a continuous mass. In the narrow spaces between them, compressed fibroblasts, numerous lymphoid cells, and mast cells are scattered. Collagenous fibers and elastic networks run in all directions between the fat cells. The argyrophil fibers are well developed, especially along the blood vessels, and form a netlike basket around each fat cell. Wassermann (1926) holds that the adipose tissue forms specific organs and that the fat cells are modified reticular cells.

The fat tissue always contains a richly developed network of blood capillaries. Because of the close relation which exists in the fetus between the developing fat tissue and the blood vessels, the fully developed fat tissue usually is more or less distinctly divided into lobules of varying size and shape, separated by partitions of fibrous connective tissue.

The most important function of the fat tissue is the storing of neutral fat. Also, because it consists of a multitude of closely packed liquid fat drops, each surrounded by an elastic membrane, the fat tissue forms soft and elastic pads between the various organs of the body.

The *brown fat tissue* must be distinguished from the common or white fat tissue. In rats and other rodents it is highly developed and forms yellowish, lobated masses in certain parts of the body—between the scapulae, on the neck, in the mediastinum, in the inguinal region, etc. Macroscopically it suggests a gland and so was called *interscapular* or *hibernating gland.* The latter name was given because this tissue was believed by some to play a peculiar rôle during hibernation.

The brown fat contains a pigment which gives the tissue its color. The fat cells are assembled in groups separated by thin networks of collagenous or reticular fibers and numerous capillaries (Fig. 64). The microscopic structure suggests an endocrine gland. While the common fat tissue loses or accumulates neutral fat with changes in the nutritional condition of the animal, these factors do not seem to affect the brown fat tissue.

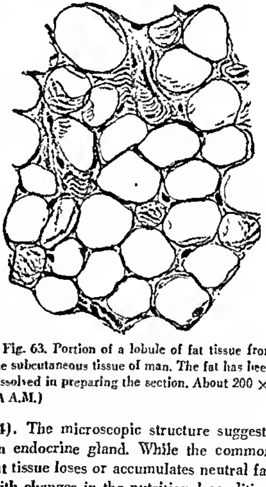

Fig. 63. Portion of a lobule of fat tissue from the subcutaneous tissue of man. The fat has been dissolved in preparing the section. About 200 ×. (A A.M.)

In adult man and other mammals the two kinds of fat tissue cannot be distinguished clearly. The morphologic interrelationships between the two and the function of the brown fat tissue require further study.

Pigment Tissue. In the lunica suprachorioidea and in the lamina fusca of the sclerae of the eye,

delicate, fibrillar networks were found in the interstitial tissue of many glands. They were called reticular or lattice fibers. The methods generally used for the demonstration of collagenous fibers do not stain them distinctly; in addition, they are too thin to be seen easily among the other elements of the tissue. They are electively impregnated with silver by modified Bielschowsky methods after which they appear as black, sharply drawn nets on a yellow or brown background (Fig. 62). This gave them the name of argyrophil fibers. Microdissection studies have shown them to be inelastic.

The reticular fibers differ chemically somewhat from the collagenous fibers. They resist digestion by trypsin in an alkaline medium as do the collagenous fibers, but do not swell on the addition of dilute organic acids. There are marked differences beween the X-ray spectrographs of the two kinds of fibers.

The fibers run irregularly in all directions; the thicker ones seem to consist of bundles of finer ones. They form networks which surround and support the various elements located in their meshes, as the hepatic cells, the uriniferous tubules in the kidney, etc.

In the blood-forming tissues the fibers of the reticulum are intimately connected with the primitive reticular cells and fixed macrophages, as they seem to be in the wall of the sinuses of the liver. In many other places in the body the fibrous reticulum is especially prominent in the neighborhood of blood vessels and is connected with the undifferentiated cells. Often, however, large stretches of reticular fibers seem to lack cells of any type. (See Chap. V.)

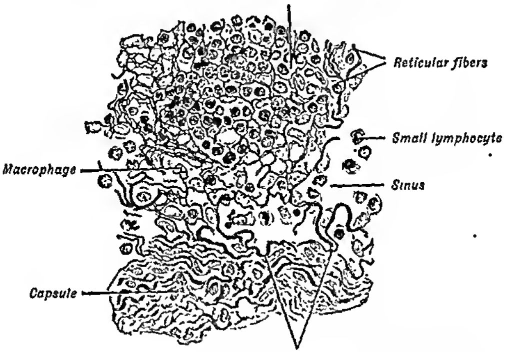

Fig. 62. A portion of the cortex with the capsule of human mesenteric lymph node. Bielschowsky stain 500 ×. (A.A.M.)

It seems doubtful that the reticular fibers are really a special type of connective tissue; frequently they continue directly into common collagenous fibers (Fig. 62).

Some claim that the argyrophil fibers are immature collagenous fibers and call them "precollagenous" fibers. The first fibers to appear when

Chapter V

BLOOD-FORMING AND DESTROYING TISSUES

The short-lived blood corpuscles are kept at a constant number in the blood by the continuous formation of new cells. Under normal conditions, blood cells are regenerated only within the lymphatic and the myeloid tissues and organs (*hemopoietic* organs and tissues). The process of their formation is called *hemopoiesis.*

The cells of the circulating blood may be divided into two groups according to their origin. To the first group belong the lymphocytes (and probably the monocytes), which originate in the lymphatic tissue and are called *lymphoid elements.* The second group consists of the erythrocytes and the granular leukocytes; these originate in the myeloid tissue and are the *myeloid elements.* But this separation of myeloid and lymphatic tissue is not absolute; for, in certain abnormal conditions in postnatal mammals, it is effaced and, in the early embryonic stages of the mammals as well as through life in most of the lower vertebrates, no such separation exists.

All of the blood cell forming tissues of adult mammals have the same fundamental structure—a fibrous and cellular stroma in the meshes of which hemopoiesis takes place. This framework is composed of reticular fibers and cells.

The reticular fibers (p. 74) are accompanied or sheathed by a thin layer of protoplasm in which are scattered pale oval nuclei. These *primitive reticular cells* often show no cell limits. They have but slight phagocytic properties and only rarely contain a few granules of waste pigment; they do not store appreciable amounts of vital dyes (Fig. 66). Like the mesenchymal cells of the embryo, they are endowed with the ability to turn into all types of blood and connective tissue cells.

From these *primitive reticular cells* there are many transitions to larger cells which are active phagocytes, called *phagocytic reticular cells* or *fixed macrophages.* These have an abundant cytoplasm, often containing waste pigment, and a large pale nucleus (Fig. 66). Most of them are stellate or spindle-shaped and adhere to the reticular fibers.

The fixed macrophages may become large *free macrophages* with thin, membrane-like pseudopodia. These cells energetically phagocytose various foreign particles which come in contact with them. They may contain débris of dead cells, engulfed erythrocytes in various stages of disintegration and, in vitally stained animals, numerous large dye granules. In fresh preparations stained supravitally with neutral red, the inclusions and vacuoles of the macrophages stain deeply.

LYMPHATIC TISSUE

In the mammals the lymphatic tissue forms distinctly outlined organs, the *lymph nodes,* which are arranged along the course of the lymph vessels. This tissue is found also in the hemal nodes and in the peripheral lymph nodules scattered in the mucous membrane of the alimentary canal and of the respiratory passages, in the conjunctiva, etc. The lymphatic tissue forms a large part of the spleen and

the majority of the cells in the loose connective tissue are melanophores. Such a tissue can be termed "pigment tissue." It is described in the section on the eye.

Connective Tissue (Lamina Propria) of the Intestinal and Uterine Mucous Membranes, the Interstitial Connective Tissue of the Lung, Testis, Ovary, etc. The connective tissue which surrounds and supports the epithelial elements in these organs acquires a specifically differentiated structure. It is described in the chapters which deal with these organs.

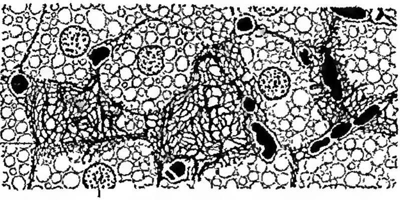

Fig. 64. Brown fat tissue from the "hibernating gland" of a white rat: *Cap*, Capillary; *d*, intercellular fibrous network. Impregnation method of Hortega. 900 ×. After Nageotte and Guyon.

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has not been proved to exist in the endothelium of the common blood or lymph vessels, the term is not justified. The flattened form of these macrophages is an adaptation to their position on the wall of the channels through which the lymph flows. The term *littoral* or *lining cells* of the system of macrophages is perhaps the best to use. (See p. 96.)

The lymphatic tissue does not contain fibroblasts (except along the arteries and veins and in the trabeculae of the lymphatic organs) although, in inflammation and in cultures of this tissue, fibroblasts develop from the primitive reticular cells and from the macrophages.

The Free Cells. The meshes of the fibrous and cellular reticulum contain free cells. In diffuse lymphatic tissue and in the nodules the free cells are so densely crowded that the nuclei of the primitive reticular cells can be seen among them only in thin sections. The free cells are much less numerous in the meshes of the sinuses; here they float in the lymph which passes slowly through the channels. Except for the free macrophages described above, the free cells are all *lymphocytes.*

In the lymphatic tissue can be distinguished several types of lymphocytes: (1) The *small lymphocytes* form the vast majority (Fig. 67). (See Chapter III.) (2) The *medium-sized lymphocytes* are scattered everywhere among the small lymphocytes, but in a much smaller number. They are slightly larger than the small variety, the nucleus is clearer and contains less chromatin; one or two nucleoli are prominent, and there is more cytoplasm Fig. 67, *b, c*). These cells divide mitotically. (3) *Large lymphocytes, macrolymphocytes.* These elements, scattered singly among the other lymphocytes, measure up to 15 or 20 μ in diameter

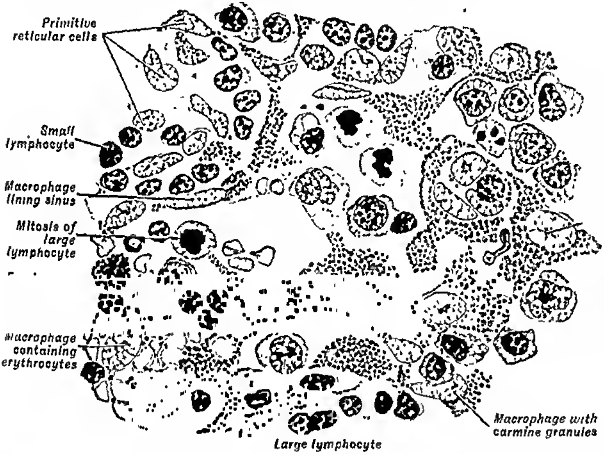

Fig. 66. Medullary sinus of mesenteric lymph node of a rabbit repeatedly injected intravenously with lithium carmine Hematoxylin-eosin azure stain 950 ×. (A.A.M.)

may be found in small amounts in the bone marrow.

Two microscopic constituents can be distinguished in the lymphatic tissue: (1) a spongelike framework or stroma (whence the name "reticular") and (2) free cells in the meshes of the stroma. These constituents are present in different proportions in various parts of the lymphatic tissue, so that we may distinguish (a) *loose lymphatic tissue*, consisting predominantly of stroma; (b) *diffuse lymphatic tissue*, in which the free cells predominate; and (c) *nodular lymphatic tissue*, a dense accumulation of fixed or free cells within loose or diffuse lymphatic tissue. Under various physiological and pathological conditions, each of these types of tissue may turn into either of the others (p. 80).

Fig. 65. From a section of a lymph node after the lymphocytes have been removed, showing the syncytium of the reticular cells and their intimate relations with the reticular fibers. Mallory-azan stain. Redrawn after Heidenhain.

The loose lymphatic tissue, as found in the lymph nodes, forms sinuses or pathways for the lymph which flows through the organ. Unlike the lymph vessels which have a free lumen and a wall of their own, the sinuses are merely portions of the lymphatic tissue which are especially loose in structure. (See Chapter XI).

The Stroma. The framework of lymphatic tissue is made up of (1) reticular fibers and (2) reticular cells (Fig. 65).

Fibers. The fibers are of the reticular type and are best shown by the silver impregnation method (p. 74). At the periphery of the nodules the framework is dense and the meshes small, while the stroma within the nodules is loose, with very thin fibers; in the loose tissue of the sinuses the large meshes are composed of coarse fibers (Fig. 70). Along the walls of all the blood vessels the reticulum is condensed.

Cells. The cells of the stroma are the primitive reticular cells and the phagocytic reticular cells or fixed macrophages. The primitive reticular cells of the lymphatic tissue have the ability to develop into phagocytes and lymphocytes, as well as into myelocytes in ectopic myelopoiesis (p. 112). Under certain conditions the fixed macrophages may become free macrophages anywhere in the lymphatic tissue; they are especially numerous in the sinuses. When the lymph contains foreign substances, such as lithium carmine or bacteria, the number of free macrophages greatly increases.

The fixed and free macrophages of the lymphatic tissue correspond closely with those of the loose connective tissue (p. 61). In the lymphatic tissue they increase by division and by development from primitive reticular cells.

The macrophages which form the walls of the sinuses, and which are attached to the fibers passing through the cavity of these spaces, are often flattened and resemble endothelial cells (Fig. 66). For this reason they have been called endothelium, but as the ability to store vital dyes and to transform into free macrophages

relatively scanty cellular and fibrous reticulum and are usually the expression of some stage of lymphocytopoietic activity which is focused at a small area in the lymphatic tissue. The structure of a lymphatic nodule is a reflection of its function and cellular constituents at a given time. The nodules appear and disappear or pass through a series of cyclic changes during which an intense new formation of lymphocytes proceeds through proliferation of pre-existing lymphocytes and to a lesser extent through transformation of the primitive reticular cells. Such lymphocytopoietic areas have been called *germinal centers.* In certain pathologic conditions, as diphtheria, burns, severe bacterial and plasmodial infections, the central portions of the nodules may consist mainly of free macrophages, reticular cells or fibroblasts. In such areas lymphocytes are not being formed; these areas have been called *reaction centers* (Fig. 68).

In a nodule which is actively producing lymphocytes the central portion may attain a diameter of 1 mm. It often has a small artery supplying it with blood. This central area in such a nodule appears paler than the surrounding mass of small lymphocytes with their dark nuclei, for the majority of its cells are medium-sized lymphocytes. They contain more mitotic figures than do the medium-sized lymphocytes in other lymphatic tissue Scattered among them are a few large lymphocytes and all transitions between the two. A few small lymphocytes also are found. Among the lymphocytes of an actively lymphocytopoietic nodule are scattered primitive reticular cells with indistinct cytoplasm (Fig. 69). These also show occasional mitoses. Macrophages with phagocytosed inclusions are distributed along the capillaries in the nodule. The peripheral margin of the nodule is often sharply outlined by densely crowded, small lymphocytes arranged in concentric layers.

In a stage of complete rest the lymphatic nodule consists mainly of small lymphocytes, so compactly arranged that the nodule stands out as a dense darkly-stained area in the diffuse lymphatic tissue.

New nodules may develop anywhere in the dense, loose, or nodular lymphatic tissue. These new areas of lymphocytopoiesis start with the appearance of many, rapidly repeated mitoses in medium-sized lymphocytes. In some instances these have been shown to arise as free cells, with a narrow rim of cytoplasm, from mitoses in the primitive reticular cells (Fig. 77). As the medium-sized lymphocytes increase in number, the pale-staining area increases in size. Many of the medium-sized lymphocytes become large lymphocytes and at this time some of the primitive reticular cells are becoming macrophages. If the new lymphocytopoietic areas develop within pre-existing nodules, the small lymphocytes surrounding the active, enlarging area are compressed to a dark-staining zone which is usually sharply separated from the pale-staining region of proliferating lymphocytes. If the new nodules develop in the diffuse or loose lymphatic tissue, they may or may not have an outer zone of small lymphocytes, depending on the density of the surrounding lymphatic tissue.

As a lymphocyte-forming nodule becomes inactive, mitoses become less numerous and the last divisions of the medium-sized cells give rise to small lymphocytes; some of the latter may possibly originate from shrinkage of the larger cells. Plasma cells also appear. The decrease of the growth pressure effaces the sharp boundary line between the center and the periphery of the nodule, which then becomes uniform in appearance and composition.

The New Formation of Lymphatic Tissue. New foci of lymphatic tissue and even lymph nodes can develop in any part of the loose connective tissue in the adult organism. When this happens, the lymphocytes and the elements of the stroma develop from the ubiquitous undifferentiated mesenchymal elements of the adult con-

when rounded. They occur everywhere, even in the sinuses, but are more numerous in the lymphatic nodules, their number varying with the functional condition of the lymphatic tissue. Very often, especially in human lymph nodes, they may be absent. Their cytoplasm forms a broad layer around the nucleus and is strongly basophil, presumably due to ribosenucleic acid. It may contain a few vacuoles at the indentation of the nucleus; the hemispherical cytocentrum with a diplosome is surrounded by a Golgi net. There are more rod-shaped mitochondria than in

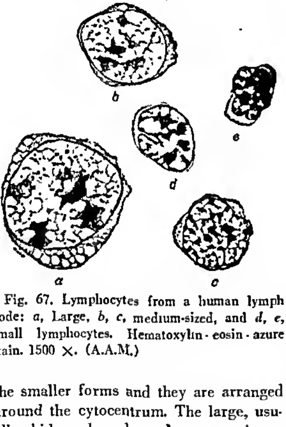

Fig. 67. Lymphocytes from a human lymph node: *a*, Large, *b*, *c*, medium-sized, and *d*, *e*, small lymphocytes. Hematoxylin - eosin - azure stain. 1500 ×. (A.A.M.)

the smaller forms and they are arranged around the cytocentrum. The large, usually *kidney-shaped nucleus occupies a* slightly eccentric position, with the excavation directed toward the large accumulation of cytoplasm. The nuclear membrane is coarsely outlined; the chromatin particles are widely scattered in a large quantity of clear nuclear sap. Always there are one or more large irregularly shaped nucleoli. The large lymphocytes are found dividing by mitosis.

These three types of lymphocytes are connected with one another by an uninterrupted series of transitional forms. In the small lymphocytes mitoses are extremely rare under normal conditions, the main source of the lymphocytes of the blood being the medium-sized lymphocyte.

Most of the evidence indicates that under suitable external conditions the small lymphocyte may hypertrophy into a larger one and regain the ability to divide. This transformation probably occurs but rarely in the lymphatic tissue; in other places in the body it has been clearly demonstrated after the small lymphocyte has circulated in the blood. It has been shown that in a case of chronic lymphatic leukemia the small lymphocytes of the blood can hypertrophy in tissue culture within a day into typical, very large lymphocytes. (See p. 107 for a discussion of the developmental potencies of lymphocytes.)

Plasma cells are of common occurrence in the lymphatic tissue, especially in the medullary cords of the lymph nodes; their number is subject to marked variation, particularly under *pathologic conditions. In some animals (rat,* mouse) plasma cells are especially numerous. Sometimes, eosinophil leukocytes are found in the lymphatic tissue. Heterophil granulocytes are a sign of an inflammatory lesion. Young forms of granulocytes (myelocytes) are found only in extramedullary myelopoiesis (p. 112). Common connective tissue mast cells are often found scattered along the fibers of the reticulum; monocytes usually do not occur.

The Development of Lymphocytes. In the postnatal mammals most lymphocytes arise by mitosis of pre-existing lymphocytes within the lymphatic tissue. This occurs mainly in the nodular but also to some extent in the diffuse and loose lymphatic tissue. The mother cell is usually *a medium-sized lymphocyte,* although dividing large lymphocytes are not uncommon. In some instances it has been possible to trace lymphocytes to their origin in primitive reticular cells—a source probably active only when the preexisting lymphocytes are unable, by their mitoses, to fill the demand for lymphocytes. (See p. 93.)

The Lymphatic Nodules. The lymphatic nodules are especially dense accumulations of lymphocytes embedded in a

nective tissue (p. 61). Also, the lymphatic tissue may undergo involution and disappear, the lymphocytes degenerating or wandering away, while the fixed reticular cells seem to be transformed into fat cells.

The Function of the Lymphatic Tissue. The most conspicuous function of the lymphatic tissue is the production of lymphocytes. The lymphocytes which are newly formed in the lymph nodes—the vast majority of them are the small variety—migrate into the sinuses and are carried away by the lymph stream into the lymphatics and the thoracic duct, and hence into the blood. In addition, numbers of small lymphocytes migrate directly from the lymphatic tissue into the blood through the endothelium of the venous capillaries. In extramedullary myelopoiesis the lymphatic tissue can also become the source of granulocytes.

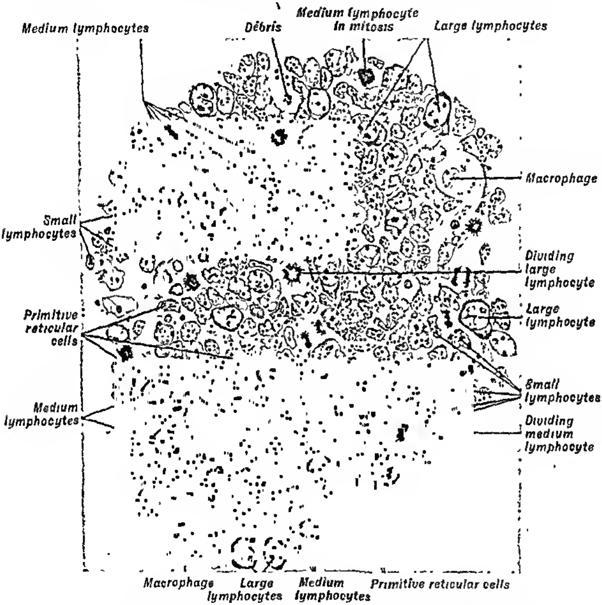

Fig. 69. Portion of an actively lymphocytopoietic nodule of a human lymph node. Hematoxylin-eosin-azure II. (A.A.M.)

In the early stages of infection of rabbits and guinea pigs with *B. monocytogenes* the lymphocytes of the lymph nodes, and occasionally even of the nodules, turn into monocytes. Lymphoid hyperplasia in malaria has been found by Taliaferro and Mulligan (1937) to have a

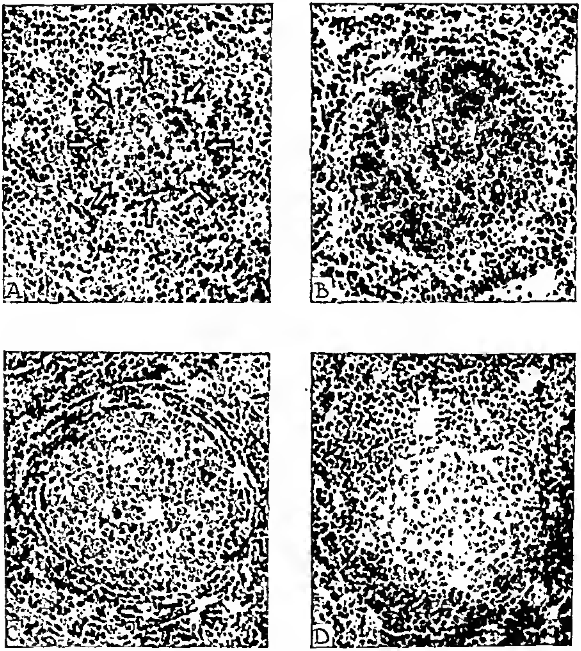

Fig. 68. Photomicrographs showing four nodules at different stages of development in mesenteric lymph node of guinea pigs, 5 days after injection of *B. monocytogenes*. *A*, Small new nodule with dividing lymphocytes. Its margins are indicated by the arrow heads. B, Later stage in the development of a lymphatic nodule. It contains 27 mitoses in medium-sized lymphocytes and lacks a peripheral zone of small lymphocytes This nodule is a typical "germinal center" of Flemming. *C*, The central portion of this nodule is actively lymphocytopoietic and contains 15 mitoses in medium-sized lymphocytes. It is demarcated from the surrounding diffuse lymphatic tissue by layers of small lymphocytes This is the type of nodule which is often regarded as typical, consisting of an outer dark-staining zone and a lighter central area *D*, This nodule has a very pale-staining central portion consisting mainly of reticular cells, a few macrophages and scattered smaller lymphocytes. This nodule with its center depleted of lymphocytes is of the "reaction center" type of Hellman. Hematoxylin-eosin-azure II. 300 ×. After Conway.

and that the delivery of lymphocytes to the circulation is, in part, under control of the adrenal cortex and of the pars distalis of the hypophysis. The source of antibodies has not been determined and the significance of the antibody content of lymphocytes awaits clarification.

The marked atrophy of lymphoid tissues which results from the action of a variety of noxious agents (part of the "alarm reaction" of Selye) is believed to result from the liberation of adrenal cortical hormones; it does not occur if the adrenal cortex is removed.

Some authors deny the importance of the nodules for the regeneration of the lymphocytes, believing them to be only centers of reaction of the lymphatic tissue to various toxic agents. As proof, they point to the degeneration of lymphocytes and of reticular cells in intoxications and burns and the inflammatory lesions which often occur in the centers, especially in infectious diseases. In this sense the nodules are reaction centers. But a careful study of the cyclic morphologic changes of the nodules clearly demonstrates that they are also germinal centers, for lymphocytes are formed more profusely in the nodular than throughout the diffuse lymphatic tissue or in the sinuses.

The lymphocytes are among the most sensitive cells in the body to ionizing radiations and certain toxins (mustard gas); the reticular cells are among the most resistant.

Embryonic lymphatic tissue has nodules which are dense masses of small lymphocytes, lacking pale-staining central areas. It is claimed that guinea pigs which have been reared for sixty days on sterile media do not develop centers in the nodules (Glimstedt).

The Lymphatic Tissue in the Lower Vertebrates. In the lower vertebrates, although lymphocytes are plentiful, their regeneration is not localized in special, lymphatic organs, but occurs in many places in the connective tissue; in fact, lymph nodes are usually absent. The most important difference in comparison with the mammals is that the lymphatic tissue is not sharply separated from the myeloid tissue.

THE MYELOID TISSUE. THE BONE MARROW

Of the several kinds of bone marrow which differ macroscopically, the two most important varieties are the *red* and the *yellow* or *fatty* bone marrow. Only the red marrow, which consists of myeloid tissue, plays a rôle in hemopoiesis, producing the red blood cells and the granulocytes. In the embryo and the newborn, red marrow only is found in the bone cavities. With progressing age the red marrow is gradually replaced by the yellow marrow with its fat cells. In the normal adult, red marrow is found in the vertebrae, the ribs, the sternum, the *diploë* of the bones of the skull, and in the proximal epiphyses of the femur and humerus. Bone marrow forms 2 to 3 per cent of the body weight.

No sharp limit can be drawn between the two kinds of bone marrow. After considerable losses of blood or in the anemias, the fatty bone marrow is replaced to a greater or lesser extent by red marrow. After prolonged starvation or in some wasting diseases the bone marrow loses its blood cells, and the fat acquires a peculiar gelatinous appearance; it is then called gelatinous or "mucous" bone marrow. In myeloid leukemia the red bone marrow becomes very rich in granulocytes and acquires a gray "pyoid" character. Figure 78 shows the effect of raising the temperature of fatty marrow.

The myeloid tissue, like the lymphatic, consists of: (1) the spongelike framework or stroma which is intimately connected with the blood vessels and (2) the free cells in the meshes of the stroma.

The Stroma. As in the lymphatic tissue, the stroma consists of primitive and phagocytic reticular cells attached to the argyrophil fibers (Figs. 65, 71). The net-

functional significance in malarial immunity in that it builds up a mesenchymal reserve from which new macrophages are with its macrophages and will be discussed with the structure of the lymph nodes and spleen.

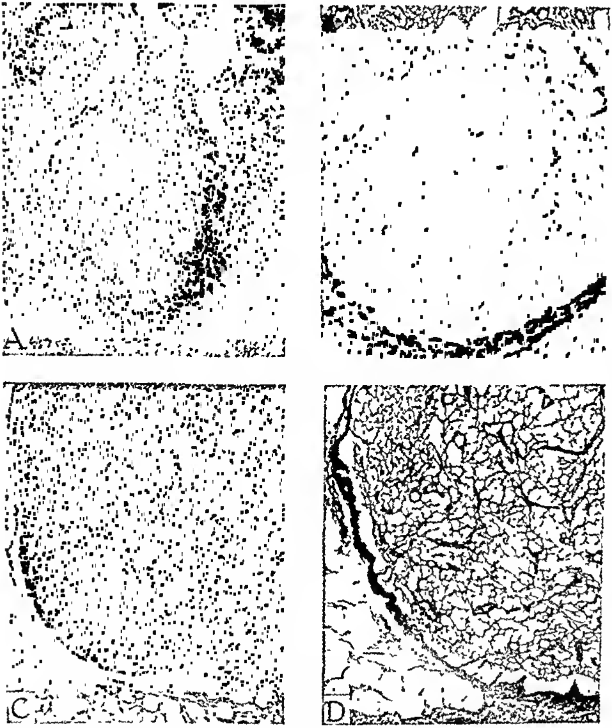

Fig. 70. A and B are photomicrographs from sections of the same block of mesenteric lymph node of rabbit, thirty-six hours after injection of *B monocytogenes*; *C* and *D*, from a block of mesenteric lymph node of rabbit, forty-eight hours after injection of the same bacterium. When nodular lymphatic tissue (A) becomes diffuse lymphatic tissue (C), the reticular-fiber framework, characteristic of the nodules (B), is lost (D). In all sections the subcapsular sinus is prominent. A and C stained with hematoxylin; B and D impregnated for reticular fibers by the Foot method. 107 ×. After Conway.

formed (p. 98). The other functions of the lymphatic tissue are concerned mainly

Evidence is accumulating that lymphocytes are rich in specific immune bodies

and veins in mammalian bone marrow require further study.

The Free Cells. In contrast to the free cells of the lymphatic tissue, those of the myeloid tissue are extremely varied in form and are scattered irregularly found everywhere between the other cells. Thus, the tissue which produces these elements always contains a ready supply of them and in case of need can forward large quantities at once into the blood (Fig. 72).

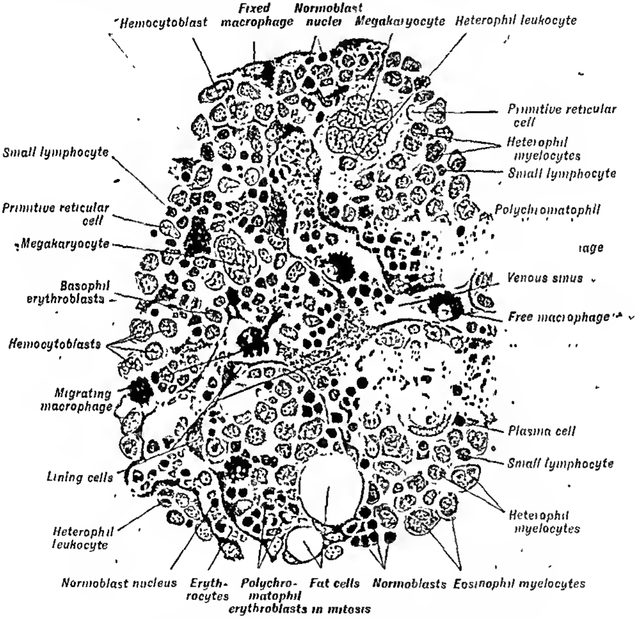

Fig. 72. Section of bone marrow of rabbit injected with lithium carmine. Hematoxylin eosin azure II. 460 ×. (A.A.M.)

throughout the tissue. The vast majority of them are young myeloid elements (erythroblasts and myelocytes).

Mature Myeloid Elements

Mature, non-nucleated erythrocytes and the three types of granular leukocytes as they occur in the circulating blood, are

Immature Myeloid Elements

The other free cells in the bone marrow are *hemocytoblasts* (*free stem cells*), *erythroblasts*—the precursors of the red blood corpuscles—and *myelocytes*—the precursors of the granular leukocytes—and *megakaryocytes.*

Hemocytoblasts. The myeloid tissue

work of cells and fibers is looser and its meshes are larger than in the lymphatic tissue. Particulate matter and vital dyes injected into the blood are taken up very rapidly by the fixed macrophages of the bone marrow.

Circulation in the bone marrow is characterized by the presence of many large vessels, called *sinusoids*, through the walls in which the arteries connect with the sinusoids needs further study.

The stroma of the myeloid tissue is distinguished by the constant presence of fat cells. These are scattered singly in the red marrow (Fig. 71), but in the yellow bone marrow they have crowded out practically all of the other cells; between them remain (besides the blood vessels

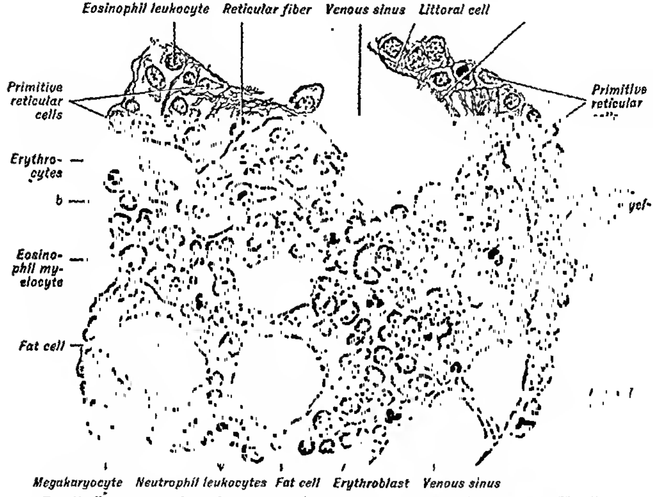

Fig 71 Bone marrow from the upper epiphysis of a femur of a child of six years. The fibrous network of the wall of a vessel is seen from the surface at *a* and in cross section at *b*. Bielschowsky stain. 500 ×. (A.A.M.)

of which innumerable cells pass into the blood stream. The sinusoids are lined by flattened, fixed macrophages (littoral cells), like those forming the walls of the lymph node sinuses These dye-storing and phagocytosing cells have indistinct limits and are in direct connection with similar cells of the stroma. They can round off and appear as free macrophages in the blood of the sinusoids. The manner and reticular fibers) only scattered fixed macrophages and primitive reticular cells. The latter are probably the main source of the new blood cells when the yellow bone marrow is transformed into red marrow. Small accumulations of lymphatic tissue along the small arteries of the bone marrow are described by most investigators, but lymph vessels have not been found. The connections between arteries

of all adult mammals contains ameboid, nongranular, basophil cells of lymphoid nature. They vary in size, the largest measuring 15 μ and are scattered singly or in groups of two or four. Their structure corresponds exactly to that of the lymphocytes. These elements are the free stem cells of all the other myeloid elements. From the unitarian point of view, they are morphologically identical with and have the same prospective developmental potencies as the larger lymphocytes. The most suitable name for them is *hemocytoblast*. The small cells of this type are considered by some to be micromyeloblasts; they are connected with the larger hemocytoblasts by a complete series of transitional forms.

Erythroblasts. The young forms of the red blood corpuscles are spherical cells with spherical nuclei and are called erythroblasts. In living cells their cytoplasm is homogeneous, and of a yellow color which intensifies as the cells develop into erythrocytes. Supravital staining with neutral red produces red precipitates in their cytoplasm. Erythroblasts never show ameboid motion. In fixed and stained sections they show mitochondria, a Golgi net and a cytocentrum. The round nucleus of the erythroblasts always presents a checker-board distribution of angular particles of chromatin. The nucleoli gradually involute. The number of mitotic divisions in the cell lineage is not known. The changes in the erythroblasts as they develop into erythrocytes are clearly shown in Fig. 73, *22–31*, and Fig. 74.

The youngest erythroblasts (those closest to the stem cell) are called *basophil erythroblasts*, because of the intense basophilia of their protoplasm (Fig. 73, *22*); it is deeper than that of the hemocytoblasts. An intermediate cell (proerythroblast, Fig. 74, *17*) has been described.

The erythroblasts of the next youngest generation have a very small amount of hemoglobin. (Some authors call them *megaloblasts*, a somewhat misleading term because it was first used for the erythroblasts of pernicious anemia which are of different nature—see Jones) After fixation and staining with the Romanowsky mixture (eosin-methylene-azure) the cytoplasm varies from a purplish-blue to lilac or gray (Fig 73, *23–27*). These erythroblasts are, therefore, called *polychromatophil*. This staining reaction is due to the appearance of the pink-staining hemoglobin in the basophil cytoplasm of the erythroblast (which stains blue with eosin-methylene-azure).

The polychromatophil erythroblasts divide mitotically. Some of them remain in the tissue in a resting condition for future use. In the others the amount of hemoglobin increases while the basophilia of the cytoplasm diminishes; in this way *normoblasts* arise in which the cytoplasm stains a bright pink with the Romanowsky mixture (Fig. 73, *29*). Normoblasts are smaller than polychromatophil erythroblasts and only slightly larger than mature erythrocytes. The small round nucleus contains a dense accumulation of angular chromatin particles and stains very dark. After an unknown number of mitotic divisions, the normoblasts lose their capacity for proliferation and their nucleus is condensed to a darkly staining body. Each mature normoblast loses its pyknotic nucleus and is transformed into a red blood corpuscle. Some investigators hold that the

Fig. 73. The development of the myeloid elements of human bone marrow from a common lymphoid stem cell, the hemocytoblast (*1, 2, 3*). *4, 5, 6*, Basophil myelocytes and in mitosis (*7*); *8*, basophil leukocytes; *9, 10, 11*, eosinophil myelocytes and in mitosis (*12*); *13*, eosinophil metamyelocyte; *14*, eosinophil leukocyte; *15*, leukoblast of Pappenheim; *16, 17, 18*, neutrophil myelocytes and in mitosis (*19*); *20*, neutrophil metamyelocyte; *21*, neutrophil leukocyte; *22*, basophil erythroblasts; *23–27*, polychromatophil erythroblasts with increasing hemoglobin content; *28*, mitosis of a normoblast; *29*, normoblast; *30*, normoblast with pyknotic nucleus, beside it an extruded nucleus; *31*, erythrocyte; *32*, two young megakaryocytes; *33*, multipolar mitosis of a megakaryocyte. The figure has been combined from sections and moist-fixed smears from fresh human bone marrow stained with hematoxylin-eosin-azure. Cells *4–7* are from moist smears fixed in absolute alcohol and stained with alcoholic thionin. The mature myeloid elements are from dry-fixed smears of normal human blood; 8 stained with alcoholic thionin, *14, 21, 31* stained with May-Grünwald-Giemsa. 1500 ×. (A.A.M.)

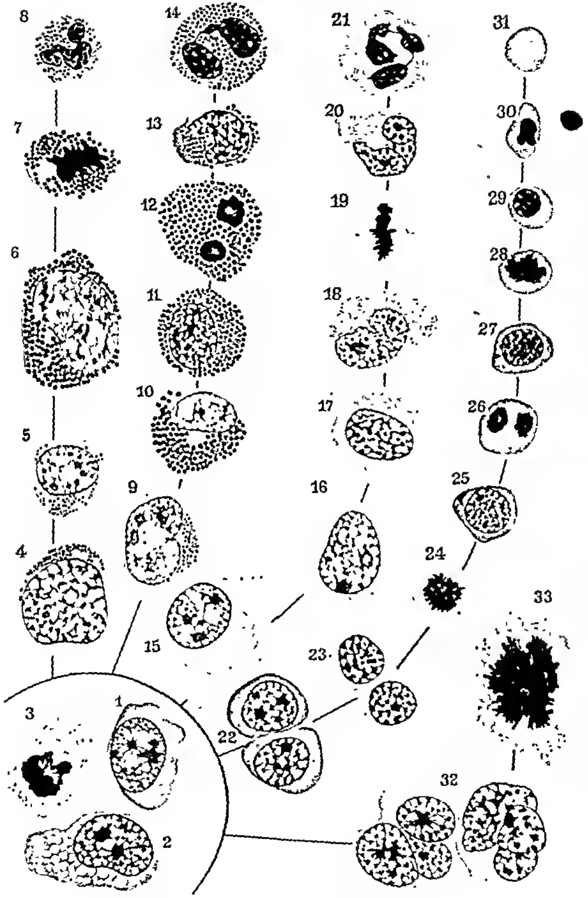

Fig. 73. See opposite page for legend.

nucleus is lost by *karyolysis*, while others believe this occurs by *extrusion*. Most of the evidence favors the latter view.

Myelocytes. Besides the erythroblasts, the young forms of the three types of leukocytes (heterophil, eosinophil, and basophil) are common cell types of the myeloid tissue. The myelocytes of each of the three types are provided with their characteristic granulation and cannot be transformed into myelocytes of another type or into elements of another kind (Figs. 72, 73). They have a compact round or kidney-shaped nucleus, and proliferate intensely by mitotic division. Some of their progeny remain unchanged while others undergo progressive maturation. Finally, each cell is transformed individually into a mature polymorphonuclear granular leukocyte. These details are clearly shown in Figs. 73 and 74 which also illustrate the differences resulting from the use of the two technics.

Myelocytes with Heterophil Granules (Neutrophil Myelocytes of Man). The heterophil myelocytes are larger than the mature heterophil leukocytes

The youngest generation is that of the *promyelocytes*. The oval or kidney-shaped nucleus contains a loose chromatin network and several nucleoli. At the indentation of the nucleus there is a distinct cytocentrum. The ameboid cytoplasm is slightly basophil, although it often shows acidophil areas The specific granules are scarce and usually are confined to the periphery of the cytocentrum and to the acidophil areas in the cell body In dry smears the promyelocytes contain, in addition to the heterophil granules, azurophil granules which later disappear.

The promyelocytes often show mitosis. In the following generation, which may be called heterophil myelocytes proper, the protoplasm becomes diffusely acidophil while the specific granules increase in number and fill the whole cell body, except for the cytocentrum (Fig. 73, *17*). The chromatin network of the nucleus becomes coarser and stains darker and the nucleoli become indistinct. Mitoses are common; during division the granules are evenly distributed among the daughter cells and continue to increase in numbers as the latter grow. Some of the heterophil myelocytes are small and have a dark nucleus; these are called *micromyelocytes*

As the result of an unknown number of mitoses, a generation of heterophil myelocytes appears which has lost its capacity for division. The nucleus in these cells, as soon as it is reconstructed after the last mitosis, shows a beginning polymorphism and has the shape of a horseshoe (Fig. 73, *20*). Such cells are called *metamyelocytes*; each of them maturates without division and is transformed by progressive constriction of the horseshoe-shaped nucleus into a mature heterophil leukocyte (Fig. 73, *21*).

Myelocytes with Eosinophil Granules. Less numerous than the heterophil myelocytes are the myelocytes with eosinophil granules, which undergo in general the same changes. Among them also different generations can be distinguished. They all have a slightly basophil protoplasm. The eosinophil promyelocytes contain a small number of specific granules which do not all stain alike. The youngest among them show a distinct basophilia and stain bluish with eosin-azure; from these there are all transitions to mature, purely eosinophil, red granules. Mitoses are common in the eosinophil myelocytes, especially in the large ones. The horseshoe-shaped nucleus of the metamyelocytes becomes constricted into two lobes in the mature leukocytes (Fig. 73, *14*)

Myelocytes with Basophil Granules.—These are much scarcer than the heterophil myelocytes and are difficult to study because their granules in man are easily soluble in water. This difficulty

Fig. 74. Cells obtained from human bone marrow as seen in dry smears, with the exception of cell *1*, which is from a dry smear of human embryonic liver. *1*, Reticulo-endothelial cell; *2*, hemocytoblast; *3*, megakaryocyte; *4*, leukoblast of Pappenheim, with azure granules; *5*, early myelocyte with azure granules and a few neutrophil granules; *6–8*, neutrophil myelocytes; *9*, neutrophil leukocyte; *10–12*, eosinophil myelocytes; *13*, eosinophil leukocyte; *14, 15*, basophil myelocytes; *16*, basophil leukocyte, *17*, proerythroblast; *18*, basophil erythroblast; *19, 20*, polychromatophil erythroblasts; *21*, normoblast; *22*, erythrocyte; *23–26*, atypical erythroblasts (megaloblasts) and erythrocyte (megalocyte) of pernicious anemia; *23*, late basophil megaloblast; *24*, polychromatophil megaloblast; *25*, orthochromatic megaloblast; *26*, megalocyte. Note differences in size of cells and in nuclear structure of the erythroblast and megaloblast series The slides from which these cells were drawn were kindly furnished by O. P Jones. May-Grunwald-Giemsa stain. 1350 ×.

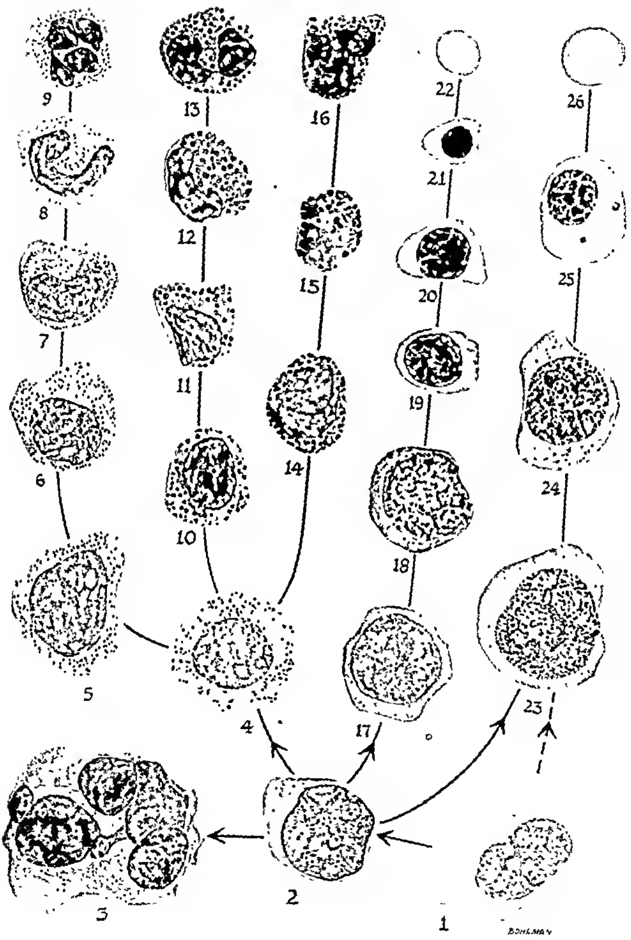

Fig 74. See opposite page for legend.

the marrow and are carried with the blood into the right heart and thence into the capillaries of the lungs, where they remain and probably undergo autolysis. Under pathologic conditions this embolism of the lung vessels by megakaryocytes may occur on a large scale; in fact not only degenerating nuclei, but even whole cells with unchanged protoplasm may be found obstructing the pulmonary capillaries. Howell finds megakaryocytes and extensive platelet-formation in the pulmonary vessels of the dog. (See also, Jordan, 1940.)

the body are increased—in the very young organism or in the adult under pathologic conditions—homoplastic hemopoiesis does not suffice, and new *erythroblasts and myelocytes develop from stem cells.* This is called *heteroplastic hemopoiesis.*

When a hemocytoblast divides, one of its various latent potencies suddenly develops and both of the daughter cells which originate from

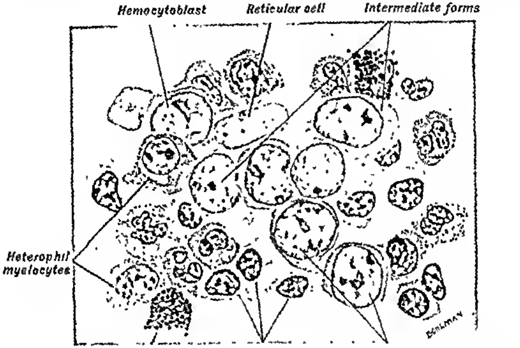

Fig. 76. Section of human bone marrow from a case of polycythemia vera showing stages in the development of primitive reticular cells into hemocytoblasts and basophil erythroblasts. Zenker-formol and hematoxylin-eosin-azure II. From a preparation of C. Huggins. 1380 ×.

Monocytes. Many hematologists believe that monocytes are formed in the myeloid tissue. Under normal conditions they are found only in the lumen of the venous sinusoids. The origin of these cells is discussed on page 108.

Heteroplastic Hemopoiesis. Under physiologic conditions, the needs of the adult organism for myeloid blood elements are usually supplied by *homoplastic hemopoiesis*—the production of mature cells by young elements of the same type. But not all of the young forms reach maturity; some of them remain unused in the tissue. Whenever the requirements of

such a mitosis show new properties. Some hemocytoblasts always remain in the tissue as the source of future hemopoietic processes when demand arises. Of the progeny of the hemocytoblasts, some become basophil erythroblasts (p. 89). These divide mitotically and are transformed in the succeeding generations into polychromatophil erythroblasts (Fig. 73, *1, 22–27*). When hemocytoblasts develop into myelocytes the two daughter cells immediately after the reconstruction of the nuclei accumulate the characteristic granules in their protoplasm (p. 91). Transitional cells between the hemocytoblasts and early myelocytes have been described under the name of *leukoblasts* (Figs. 73, 74). Their structural differences are so insignificant that to recognize these elements as a separate cell type seems unwarranted.

induced some investigators to declare that cells with basophil granules in the bone marrow are degenerated eosinophil myelocytes. For the most part the basophil myelocytes are small cells with a paler nucleus than other types of myelocytes. The protoplasm contains a widely varying number of specific, basophil, metachromatic granules of unequal size. Mitoses have been found but are very rare (Fig. 73, 7).

Megakaryocytes. These giant cells with a polymorphous nucleus are characteristic of the mammalian bone marrow, where they are scattered evenly among the other elements. Some of them have a diameter as large as 40 μ. The form of the cell body is spherical, but its surface is often provided with irregularly shaped processes.

The nucleus is deeply constricted in many places; the lobes bulge at the periphery, while their central parts are all interconnected by short, branched stalks (Fig. 75). The interior of the nucleus shows a chromatin network and indistinct nucleoli. In the living cell the abundant cytoplasm is homogeneous and contains many groups of centrioles which are scattered in the furrows of the nuclear surface. Mitochondria and a Golgi net have also been described. With special fixation and staining, fine, azurophil granules are seen to be distributed in the cell body in large quantities, sometimes in small dense groups. The presumed rôle of the megakaryocytes in the production of platelets is discussed on page 53.

In every normal bone marrow many megakaryocytes are found degenerating (Fig. 75, *b*) while there are frequent signs of their new formation from hemocytoblasts.

The first stage of this transformation consists of a hypertrophy of the growing nucleus which then becomes constricted in several places. Then follows a series of peculiarly modified, mitotic divisions which concern only the nucleus. The centrioles divide into several groups and a complex spindle with several poles arises. The chromosomes are arranged in several equatorial planes and give rise to several daughter nuclei. There is no constriction of the cytoplasm. Instead, the daughter nuclei at once fuse into a new, larger nucleus which contains more chromatin than the mother nucleus. After an unknown interval a new mitosis with still more numerous centers occurs, the daughter nuclei again fuse in the telophase, and the quantity of chromatin and the number of the centrioles again increase. The number of succeeding mitoses is not known. Sooner or later the cell degenerates; the giant nucleus shrinks, the cytoplasm disintegrates and the final result is a naked, shrunken, nuclear remnant (Fig. 75, b). Such degenerated nuclei often find their way into the sinusoids of

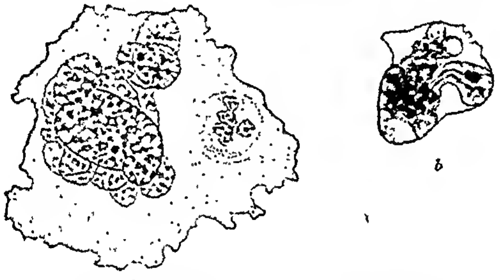

Fig. 75. *a*, Megakaryocyte containing a neutrophil leukocyte; *b*, degenerating megakaryocyte; both from human bone marrow. 1500 ×. (A.A.M.)

and bone marrow the red blood cells develop extravascularly, while in the yolk-sac they are preponderantly of intravascular origin.

Functions of the Myeloid Tissue. The main function of the bone marrow seems to be the production of myeloid elements for the blood. The macrophages of the bone marrow also function like the macrophages in other tissues (see p. 96).

percentage of myelocytes of this type increases greatly. In certain other conditions, as in typhoid fever or in agranulocytic angina, the heterophil myelocytes decrease in number. Whenever there is an increased need of erythrocytes or where the erythrocytes are destroyed in large quantities, they and their precursors predominate in the myeloid tissue. That temperature plays some rôle in the control of

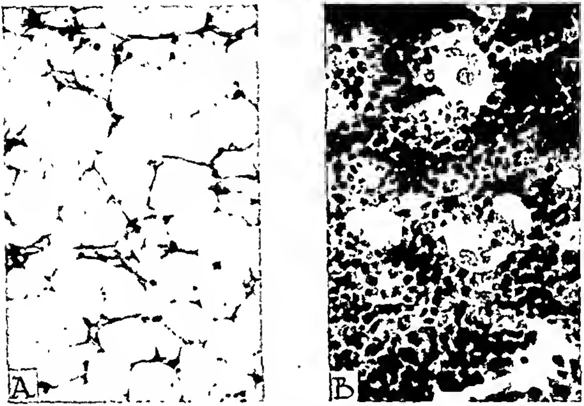

Fig. 78. A tail loop was constructed in a twenty-three-day rat, by skinning the distal half of the tail and inserting it surgically in the peritoneal cavity, where it was kept for one hundred and twenty-five days. The section on the left (A) is from the cool outside loop and shows fatty bone marrow; that on the right (B) shows hemopoietic marrow from the warm region of the tail in the abdominal cavity. 500 ×. Courtesy of C Huggins.

The cellular composition of the blood is intimately connected with the condition of the bone marrow. Under physiologic conditions, the relative numbers of the different cells in the bone marrow, as in the blood, vary only a little. But all general pathologic processes immediately affect the composition of the bone marrow. In most general infections, during suppuration, etc., the heterophil granulocytopoietic appartus is stimulated and the

hemopoiesis in the marrow is shown by the fact that the fatty marrow of the tail bones of a rat becomes hematopoietic when the temperature of the bone is raised to that of the body, as by placing it in the body (Fig. 78, *a*, *b*).

The production of erythrocytes depends in part on an *anti-anemic factor* which is stored in the liver. This factor probably is the result of the interaction of a substance in the gastric juice (*intrinsic fac-*

The New Formation of Free Cells from Fixed Cells. In the embryo the hemocytoblasts of the bone marrow which produce the young myeloid forms originate from undifferentiated, fixed mesenchymal cells. In the adult the same process may occur. It has been explained that some of the cellular elements of the reticulum always remain undifferentiated. But under physiologic conditions it is rare for primitive reticular cells to become free, basophil hemocytoblasts in the bone marrow. The mitoses of the myelocytes and erythroblasts—and occasionally of hemocytoblasts—usually are sufficient. Pathologic stimuli sometimes facilitate the new formation of hemocytoblasts from the primitive reticular cells (Fig. 76). As described on page 80 heteroplastic development of lymphocytes may also occur (Fig. 77).

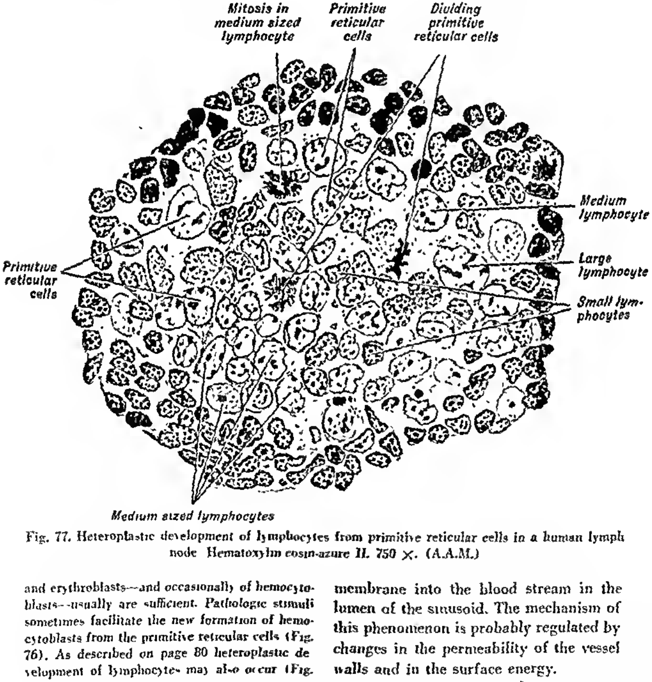

Fig. 77. Heteroplastic development of lymphocytes from primitive reticular cells in a human lymph node Hematoxylin eosin-azure II. 750 ×. (A.A.M.)

How the Myeloid Elements Enter the Blood. Because the myeloid elements arise outside the blood stream, it is obvious that the newly formed. mature myeloid cells must pass through the walls of the blood vessels to enter the circulation. The very thin-walled venous sinusoids make this possible. Through them easily pass, not only the ameboid mature granular leukocytes, but also the non-motile erythrocytes. When these are ready for circulation they slip through the membrane into the blood stream in the lumen of the sinusoid. The mechanism of this phenomenon is probably regulated by changes in the permeability of the vessel walls and in the surface energy.

The claim that red blood corpuscles in the adult, normal man are formed intravascularly is based on unconvincing evidence. In the embryonic mammalian liver

"reticulo-endothelial system," as defined by Aschoff.

The most easily controlled criterion for deciding whether a cell of the connective tissue or blood is a macrophage is the elective storing of colloidal vital dyes (Fig. 79); that is, the cell in question must accumulate the dye from solutions so weak that the other elements of the connective tissue do not take up appreciable amounts of it. Any cell surrounded by a concentrated solution of a colloidal dye may become filled with dye granules. Macrophages in tissue culture take up droplets of water by *pinocytosis* (W. Lewis).

The macrophages may be found as fixed, nonmotile elements, or as rounded, ameboid cells. They can adapt themselves structurally to a peculiar position in the tissue, as in the thin cells lining the sinuses in the lymph nodes, bone marrow, liver, and spleen.

All fixed macrophages have the potency of wandering under the influence of certain stimuli. In the spleen and liver they phagocytose worn-out or damaged erythrocytes, with the result that iron-containing pigment may accumulate in their cytoplasm. Fat and lipoid inclusions are common.

Macrophages play an *important rôle* in the general metabolism and in the so-called general and local "defense" reactions. The storing of vital dyes is a special type of "defense" activity. The ultramicroscopic particles of the colloidal solution enter the cell body in an invisible manner and aggregate in the cytoplasm into large particles. The particles then are gradually destroyed, at least in part through intracellular digestion; in this way the organism gets rid of some of the foreign substance. Although macrophages wander under the influence of certain stimuli and their precursors are often distributed by the blood stream, they tend to remain in local sites with the result that many "defense activities" are localized. For example, dust and other materials entering the lungs are removed by the so-called septal cells; other macrophages of the body have no chance to phagocytose this material. Foreign particulate material in the blood stream is removed by the macrophages in such organs as the liver,

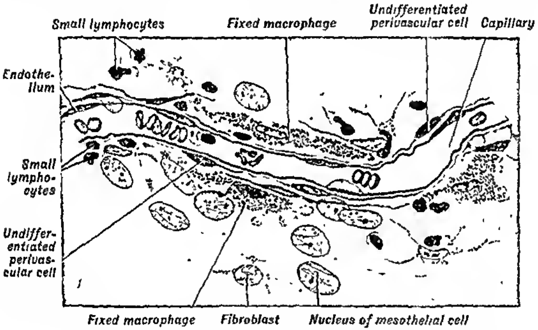

Fig. 79. Stretch preparation of omentum of a rabbit vitally stained with lithium carmine. Hematoxylin stain. 500 ×. (A.A.M.)

tor of Castle) with some substance in the diet (*extrinsic factor*). The absence of the anti-anemic factor results in pernicious anemia, which can be treated successfully by the administration of liver or gastric extract.

The erythroblasts are about as sensitive to ionizing radiations as the hemocytoblasts (lymphocytes). The myelocytes are more resistant and the megakaryocytes much more so, while the reticular cells are extremely resistant.

The curious phenomenon of bone marrow embolism is explained by the extreme permeability of the walls of the venous sinuses. Mechanical injury, or even the breaking of a bone, or such influences as an intravenous or intraperitoneal injection of ground liver tissue, not only causes more megakaryocytes to be transported from the bone marrow to the lungs, but also numerous other cells and even large pieces of bone marrow tissue, with a subsequent plugging of the pulmonary vessels.

THE SPLENIC TISSUE

The spleen contains two kinds of tissue, the white pulp and the red pulp. The white pulp, which accompanies the arteries, is lymphatic tissue. The red pulp, a modified lymphatic tissue, is pervaded by countless, thin-walled, venous sinuses and consists of a stroma which enmeshes free cells. The stroma is like that of the lymphatic and myeloid tissues, and the fixed macrophages which line the venous sinuses can be grouped, with the flat cells of the lymphatic sinuses in the lymph nodes and venous sinuses in the bone marrow, with the lining cells of the macrophage system (Fig. 243). The red pulp receives its peculiar character from its free cells and the intricate connections between its arterioles and venules (See chapter on the Spleen.)

The most prominent of the free cells in the red pulp are the erythrocytes and leukocytes of the circulating blood. The erythrocytes give the tissue its red color. The ever-present free macrophages often contain phagocytosed erythrocytes and granules of hemosiderin and waste pigment. The majority of the leukocytes are small, medium-sized, and large lymphocytes, identical with those in the white pulp. Nearly always there are some monocytes; their number may occasionally be quite high. Countless transitional forms between the lymphocytes and the monocytes are also present. In extramedullary myelopoiesis, especially in myeloid leukemia, the meshes of the red pulp contain myelocytes of the three types and erythroblasts; they also occur in the sinuses.

THE MACROPHAGES HISTIOCYTES; RETICULO-ENDOTHELIUM

Certain cells scattered all over the body have the ability to take up particulate matter and to store foreign substances brought to them in colloidal solution. These cells are the *macrophages* (clasmatocytes or resting wandering cells) of the loose connective tissue, the *reticular cells* of the lymphatic and myeloid tissues, the *v. Kupffer cells* in the sinuses of the liver, lining cells of the sinuses in the adrenal and hypophysis, and the *adventitial cells* about the blood vessels. The "dust cells" of the lungs are included among the macrophages because they can take up particles of dust brought in with the inspired air.

All of these phagocytes, although frequently dissimilar under physiological conditions, react similarly in response to the same stimuli. This observation led to the idea that they constitute a single class of cells, to which a variety of names has been applied. Because Metchnikoff, who called them macrophages, was the first to recognize that they belong to a single physiological system and to see clearly their function in inflammation and immunity, the term macrophage system is probably the most appropriate for this "system" of cells. So defined, the macrophage system is essentially the same as the

marrow in a wide variety of animals. Some of them connected this destruction of erythrocytes with the formation of bile pigment; the erythrophagocytosis was correlated with the high iron content in the spleen (v. Ebner). The rôle of the macrophages, especially of the v. Kupffer cells in the liver of the goose, in the normal destruction of erythrocytes with the retention of the iron in the phagocytes and the elimination of the bile pigment from these cells has been clearly shown (McNee, 1913). (See review by Rous.) In certain pathologic conditions when the erythrocytes degenerate in large quantities, erythrophagocytosis in the spleen is greatly increased and the cytoplasm of the phagocytosing macrophages contains large amounts of hemosiderin and waste pigment. The red pulp of such a spleen acquires, macroscopically, a distinct brown color. In these disturbances, other organs, especially the liver with its Kupffer cells, may also take part in the destruction of the erythrocytes.

However, the phagocytosis of whole erythrocytes is probably not the only way that worn-out cells are disposed of under normal conditions. A constant disintegration of erythrocytes into small, hemoglobin-stained fragments in the circulation itself is thought to be an important factor. The fragments are taken up by the macrophages in various regions of the body and the hemoglobin is broken into hematin and globin. The first is further split into bilirubin, which is excreted with the bile, and into iron, which is retained, especially by the spleen, and utilized for the formation of new erythrocytes. How this iron is transferred from the spleen to the bone marrow has not been described.

The presence of degenerating leukocytes in the circulating blood, although often described, has never been confirmed conclusively. Destruction of granular leukocytes through phagocytosis by the Kupffer cells in the liver has been observed. Large numbers of lymphocytes may degenerate in the very place where they are formed in the lymphatic tissue (p. 81). In addition, the organism always loses lymphocytes through migration into the cavity of the intestine. It has been estimated that the life of a lymphocyte in the circulation is about 12 hours.

EMBRYONIC DEVELOPMENT OF BLOOD AND CONNECTIVE TISSUE

The manner in which blood cells develop in embryonic and postnatal animals is one of the most controversial subjects in histology. In our opinion, the following presentation is the most accurate, although some hematologists do not agree. Downey's Handbook of Hematology contains extended discussions of the subject.

Blood is formed in practically the same manner in all embryonic mammals. Beginning hemopoiesis is the same in almost all situations and consists in the rounding up of outstretched mesenchymal cells into free basophil cells which in turn give rise to all types of blood cells. The first site of this process is the wall of the yolk sac, succeeded by the body mesenchyme, liver, bone marrow, spleen, and lymph nodes. In the yolk sac most of the *primitive stem cells* become *primitive red blood corpuscles* which serve as oxygen carriers until they are replaced by the permanent erythrocytes. The remaining stem cells give rise to the *definitive* or *permanent red blood cells, granulocytes, and megakaryocytes.* In all of the other situations in which blood formation occurs, the process is the same except that primitive erythroblasts are not formed.

In all areas of embryonic blood formation the free stem cells are morphologically the same as the various-sized lymphocytes (or hemocytoblasts) of the adult. Even in the primordia of the lymphatic tissue large numbers of erythrocytes, myelocytes, and megakaryocytes are formed. It is only in the late embryonic stages that

spleen and bone marrow where they come in contact with the blood. Similarly, foreign particulate material in the lymph stream is removed by the macrophages of the lymph nodes.

Macrophages filled with a foreign substance become less capable of performing other functions, such as, perhaps, the elaboration of antibodies. If the macrophages have taken up indigestible particulate matter, as colloidal silver or India ink, they often degenerate and so set free the foreign particles, which may be taken up again by other macrophages. In the intestines and in the lungs the foreign particles may be eliminated from the body with the cells containing them.

Although most investigators believe that macrophages produce antibodies, Ehrich and Harris question this conclusion.

In any type of connective tissue, the fixed macrophages are transformed into free *inflammatory macrophages* or *polyblasts* under local inflammatory stimuli. The same is true of the fixed macrophages in a fragment of any tissue which is cultivated outside the body. In the omentum, under physiologic conditions, large numbers of such cells are set free and get into the serous exudate (see p. 69). In animals which have been repeatedly injected with adequate doses of a vital dye, and in general infections, large numbers of free macrophages are mobilized in the spleen, liver, and bone marrow (Fig. 72). Some of them get into the lumen of the venous sinuses and are carried with the blood into the right heart and on into the capillaries of the lungs, where most of them are filtered off. Only a few seem to pass into the general circulation. Some investigations indicate that they appear in the blood only during agony.

Many authors assume that new macrophages arise only from the mitotic proliferation of existing macrophages. This is not correct. In considering the source and behavior of macrophages in defense it must be pointed out that they cannot be sharply separated from the lymphoid cells. It seems probable that in all defense reactions some new macrophages arise locally from the mitotic division of preexisting macrophages or by the direct assumption of phagocytic activity by cells having mesenchymal potencies. In the skin the latter are the outstretched perivascular mesenchymal cells; in the myeloid tissues they are the primitive reticular cells. In addition many new macrophages arise from the hypertrophy and development of lymphocytes and monocytes. In the skin these latter are hematogenous; in the spleen and other reticular tissue they represent the lymphocytes which actively proliferate under the stimulus of inflammation. In order to signalize the fact that macrophages may develop homoplastically from macrophages and heteroplastically from lymphoid cells (and primitive reticular cells), Taliaferro and Mulligan proposed the term lymphoid-macrophage system to include both macrophages and all macrophage precursors.

According to Chèvremont, macrophages do not constitute a specific cell lineage; rather they represent a functional transformation of many different types of cells under the influence of cholin. The question of whether the macrophages have hemopoietic potencies is discussed on p. 114.

The Destruction of Blood Corpuscles. Both the red and the white corpuscles constantly perish in large numbers, even under normal conditions. With the aid of isotopes it has been shown that the life span of a human erythrocyte is about 120 days. The manner and site of the physiologic destruction and final disposal of the erythrocytes have not been completely elucidated. The older histologists and pathologists noted the frequent destruction of red blood cells in the phagocytes of the spleen, liver, and bone

vessels produce a few heterophil and eosinophil granulocytes. The hemopoietic activity of the yolk sac in man continues but a short time and the organ soon atrophies. When the yolk sac of the rat is transplanted to the anterior chamber of the eye, the hemocytoblasts produce great numbers of myelocytes instead of primitive erythroblasts (Block).

sides, but these same elements in the specialized blood-forming areas of the mesenchyme are the source of a most intense hemopoiesis.

Vascular Endothelium. In embryonic early stages, the endothelium of the blood vessels is identical in its potencies with the common mesenchymal cells. Thus, in the yolk sac vessels and in the caudal portion of the aorta, the endothelial

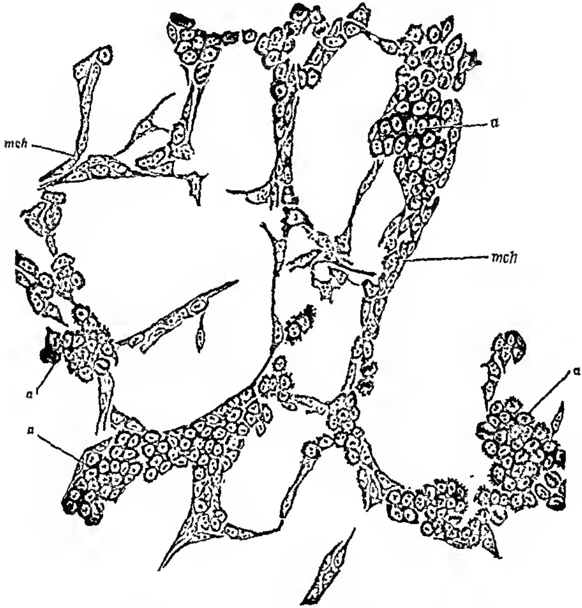

Fig. 81. Stretch preparation of the wall of the yolk sac of a guinea-pig embryo of thirteen days. Development of blood islands, *a*, from the cells of the peripheral, mesenchymal mesoblasts, *mch*. Eosin-azure stain. 220 ×. (A.A.M.)

Body Mesenchyme. In the diffuse mesenchyme of the body, wandering cells of hemocytoblastic and macrophage appearance occasionally give rise to small extravascular groups of myelocytes and erythrocytes; most of these degenerate. The hemopoietic activity of the wandering cells in the diffuse mesenchyme soon sub-

cells form clusters of hemocytoblasts. The endothelium of the vessels of the embryonic liver, bone marrow, and spleen may take part for a short time in the production of hemocytoblasts. Later, this endothelium becomes the littoral cells of the macrophage system which either have lost or do not use their hemopoietic powers in the

an apparent division of blood-forming tissues into myeloid and lymphatic takes place, and this division seems to hold for most of the normal adult life. Under abnormal conditions, however, the myeloid potencies of the cells of the lymphatic and loose connective tissues may become apparent even in the adult mammalian organism. *Blood formation in the embryo*

Fig. 80 Mesenchyme from the head of a rabbit embryo of nine and three-quarter days. Development of large lymphoid wandering cell (*Lm*) from mesenchymal cells (*M* and *M'*). Eosin-azure stain. 1025 ×. (A.A.M.)

thus takes place through the development of a hemopoietic tissue whose constituent cells are qualitatively the same but which vary quantitatively in the successive locations in which this process takes place.

Origin of Mesenchyme. The mesenchyme arises from the mesoderm through the isolation from this layer of cells which become distributed singly and in groups in the spaces between the three germinal layers. The sclerotomes are an especially abundant source of the mesenchyme. Some mesenchymal cells also arise from the surface of the parietal mesoderm facing the ectoderm, from the surface of the visceral mesoderm facing the entoderm, and from the lateral layer of the somites, the skin plate.

Yolk Sac. In early human ova irregular strands of primitive mesodermal cells traverse the small chorionic "cavity." As fluid accumulates in the blastocyst, these strands cover the surfaces of the chorionic, amniotic and yolk sac vesicles. As the embryo develops, the yolk sac becomes larger and its mesoderm assumes a more typical epithelium-like arrangement. This yolk sac mesoderm is apparently the source of the yolk sac mesenchyme which then develops hematopoietically as in other mammals. The mesenchyme between the splanchnopleure and the entoderm gives rise to groups of spherical basophil cells (the *blood islands*) connected with one another by strands of elongated cells (Fig. 81). The peripheral cells of the islands and those of the strands become transformed into endothelial tubes. The endothelium secretes the blood plasma, which fills the tubes. In this way the first blood vessels, the yolk sac vessels, arise. The round cells of the islands are the first blood cells. In the first stages of development, the endothelial cells of the blood vessels in the area vasculosa are often seen to swell, and become free in the lumen as additional primitive blood cells (Fig. 82).

The first blood elements are hemocytoblasts. They are free mesenchymal cells and are usually called *primitive blood cells* (Fig. 82). Almost immediately after their formation most of them elaborate hemoglobin and become *primitive erythroblasts* (Fig. 83). They accumulate large quantities of hemoglobin and finally cease dividing, although the nucleus remains in the cell. Such older forms are called *primitive erythrocytes;* they serve the growing embryo as oxygen carriers, but finally die out. They do not form definitive erythrocytes. A few of the primitive blood cells remain unchanged as ameboid basophil hemocytoblasts (Fig. 83). The intravascular hemocytoblasts at these early stages sometimes form atypical megakaryocytes. Free phagocytes arise from the primitive endothelial cells and are shed into the lumen. These are the first macrophages of the embryo; they engulf degenerating blood cells. In the human yolk sac vessels, the hemocytoblasts later produce a few secondary erythroblasts (Fig. 84) identical with those in adult bone marrow.

The primitive wandering cells (hemocytoblasts) in the mesenchyme outside the yolk sac

Fig. 83 Two sections through folds of the wall of the yolk sac of twenty-four-day human embryo (H1516 Univ. Chicago Emb. Coll.). *A*, Early stage of hematopoiesis, consisting of proliferating extravascular hemocytoblasts (*1*, *1'*). *L* is the lumen of a small vessel containing a few primitive polychromatophil erythroblasts. *B*, Later stage of hematopoiesis showing transformation of hemocytoblasts (*1*) into primitive basophil erythroblasts (*1a*), primitive polychromatophil erythroblasts (*2*, *3*), and primitive erythrocytes (*4*). *5*, Mesenchymal cells; *6*, endothelium; *Spl. mes*, splanchnic mesothelium Hematoxylin eosin-azure II. 1100 ×. (From Bloom and Bartelmez, The American Journal of Anatomy Vol. 67, No. 1, July, 1940.)

adult organism. In all the other vessels, the endothelium loses its hemopoietic potency very early (Chapter X).

Liver. The liver, the second blood-forming organ of the embryo, develops as a network of branching epithelial strands from the epithelium of the intestine. Large, thin-walled blood vessels are located in the meshes of this network from the very beginning. Between this endothelium and the epithelium are thin layers of mesenchyme which give rise to hemocytoblasts. They proliferate hemopoietically. The liver cells are soon outnumbered by the dense masses of extravascular definitive erythroblasts, a few megakaryocytes and myelocytes are also present.

The erythroblasts produce mature erythrocytes which slip through the walls of the sinusoids and enter the general circulation The endothelium of these vessels is transformed into a layer of macrophages which form the Kupffer cells of the adult. Toward the end of gestation the hemopoietic activity of the liver gradually subsides so that only small foci of erythroblasts can be found in the liver of the newborn. These, too, soon disappear.

Bone Marrow. The third hemopoietic organ of the mammalian embryo is the bone marrow. The myeloid tissue develops from the primitive bone marrow, the mesenchyme which resorbs the cartilage in the bones of endochondral origin and fills the spaces between the bone trabeculae of the endochondral or periosteal bone. Here again, the process is the same in principle as in the diffuse mesenchyme of the body and in the liver. Some of the fixed mesenchymal cells become wandering cells of hemocytoblastic or macrophage type. These proliferate and form dense, extravascular clusters of erythroblasts, groups of myelocytes of the three different types, and megakaryocytes. Soon, especially in the older regions, as in the diaphyses of the long bones, a solid mass of myeloid tissue develops. Of the original fixed mesenchymal elements, only a few stellate cells remain between the meshes of the young blood cells; some of them remain as the primitive reticular cells of the stroma of the bone marrow while the others develop into macrophages and fat cells. Argyrophil fibrils develop about them. The primitive endothelium of the vessels becomes the littoral macrophages in later stages.

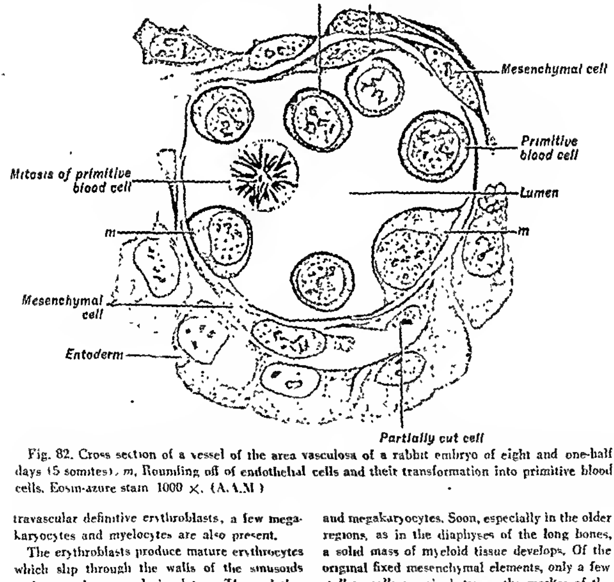

Fig. 82. Cross section of a vessel of the area vasculosa of a rabbit embryo of eight and one-half days (5 somites). *m*, Rounding off of endothelial cells and their transformation into primitive blood cells. Eosin-azure stain 1000 ×. (A.A.M.)

The Lymphatic Organs. The lymph nodes

in the adult connective tissue. Most of them, however, become fixed macrophages. The primitive wandering cells also give rise to mast cells, which then proliferate mitotically.

The appearance of the primordia of the white and of the brown fat tissue is closely connected with the development of networks of blood vessels. The fibrillar intercellular substance of the connective tissue around the growing capillaries undergoes a peculiar dissolution and the mesenchymal cells in these areas proliferate and form loose, cellular networks. Although some consider such accumulations of cells as specific, primitive, fat organs, it is more probable that these elements are common mesenchymal cells, which accumulate fat droplets and become fat cells. In the primordia of the brown fat tissue stellate cells assume a polyhedral form and the accumulated fat droplets fail to fuse.

Fig. 85. The development of reticular fibers in a twenty day culture of adult rabbit thymus. The reticular fibers stain black. Bielschowsky-Foot and Mallory-Azan stains. 900 ×. (A.A.M.)

The Origin of Fibers. According to some investigators, fibers develop through a direct transformation of living substance of the cells. But most authors believe they arise between the cells through a condensation or crystallization of an intercellular substance secreted by the cells.

The process of collagen formation is identical in principle in the body of an embryo, in young scar tissue and in a tissue culture; the silver impregnation meth-

arise along the course of the lymphatics or in the walls of the primitive lymph sacs in relatively late stages of embryonic development. Here again, in circumscribed areas of the diffuse mesenchyme, many fixed mesenchymal cells are transformed into wandering cells. As in the other blood forming organs cells of hemocytoblastic and free macrophage types can be distinguished (Fig. 238). Wandering cells of the small lymphocyte type, rarely found in the bone marrow, now appear in large numbers. The number of large and small lymphocytes increases, in part, through

Thus, in the embryo of the mammals the myeloid and the lymphoid elements are not sharply separated from each other.

In the *spleen* the lymphocytes develop in much the same way as in the lymph nodes, although more erythrocytes and other myeloid cells are formed. Some myelocytes also develop from the lymphoid wandering cells in the embryonic thymus.

Loose Connective Tissue. When connective tissue fibers appear in the mesenchyme, this tissue becomes the connective tissue. The exact

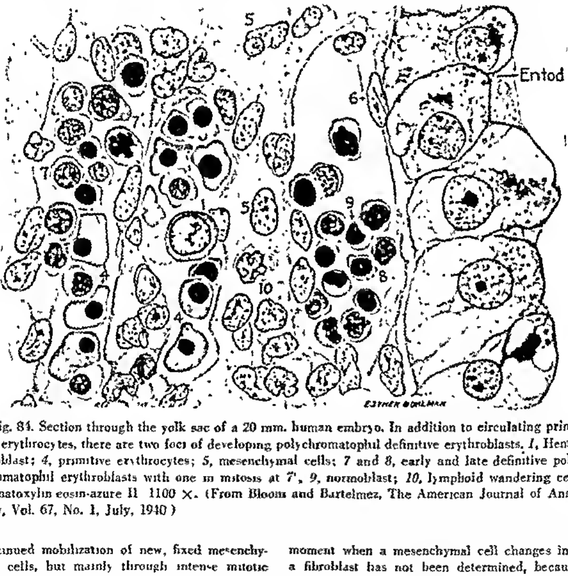

Fig. 84. Section through the yolk sac of a 20 mm. human embryo. In addition to circulating primitive erythrocytes, there are two foci of developing polychromatophil definitive erythroblasts. *1*, Hemocytoblast; *4*, primitive erythrocytes; *5*, mesenchymal cells; *7* and *8*, early and late definitive polychromatophil erythroblasts with one in mitosis at *7'*, *9*, normoblast; *10*, lymphoid wandering cell. Hematoxylin eosin-azure II 1100 ×. (From Bloom and Bartelmez, The American Journal of Anatomy, Vol. 67, No. 1, July, 1940.)

continued mobilization of new, fixed mesenchymal cells, but mainly through intense mitotic proliferation of the free lymphocytes. The fixed mesenchymal cells which remain between the free cells become the cellular components of the reticular stroma, and in later stages elaborate argyrophil fibrils.

The lymphatic tissue in the embryo always contains many heterophil and eosinophil myelocytes and a few megakaryocytes and erythroblasts; these develop from the same wandering cells from which the small lymphocytes arise.

moment when a mesenchymal cell changes into a fibroblast has not been determined, because there is no appreciable change in structure. In fact, in all regions of the body some fixed mesenchymal cells remain undifferentiated, mainly along the capillaries (p. 61).

At the later embryonic stages the vast majority of the wandering cells in the connective tissue are macrophages; hemocytoblasts are rare except in the primordia of the lymphatic organs and the bone marrow.

Many of the wandering cells persist as such

in the adult connective tissue. Most of them, however, become fixed macrophages. The primitive wandering cells also give rise to mast cells, which then proliferate mitotically.

The appearance of the primordia of the white and of the brown fat tissue is closely connected with the development of networks of blood vessels. The fibrillar intercellular substance of the connective tissue around the growing capillaries undergoes a peculiar dissolution and the mesenchymal cells in these areas proliferate and form loose, cellular networks. Although some consider such accumulations of cells as specific, primitive, fat organs, it is more probable that these elements are common mesenchymal cells, which accumulate fat droplets and become fat cells. In the primordia of the brown fat tissue stellate cells assume a polyhedral form and the accumulated fat droplets fail to fuse.

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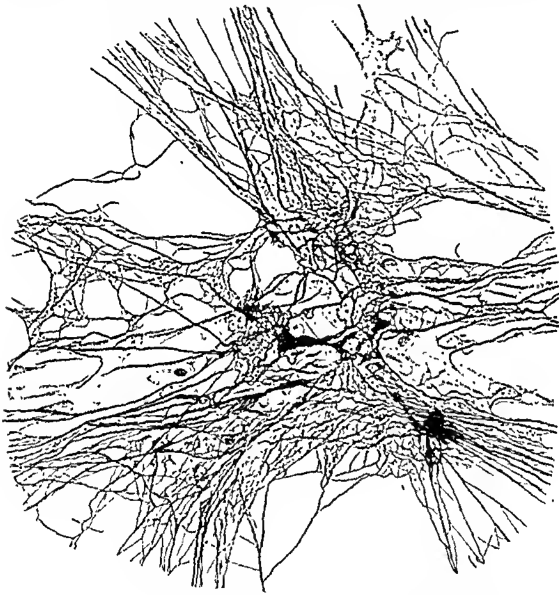

Fig. 85. The development of reticular fibers in a twenty-day culture of adult rabbit thymus. The reticular fibers stain black. Bielschowsky-Foot and Mallory-Azan stains. 900 ×. (A.A.M.)

ods give a clear insight into the morphology of this process. Delicate networks of fine, branching and anastomosing argyrophil fibrils appear on the surface and between the fibroblasts (Fig. 85). The fibrils may follow the outlines of the cell bodies and their processes, but they also extend far into the intercellular substance (nutritive medium in the case of the cultures). The first networks increase in number and thickness, and then are rearranged into parallel, wavy bundles which con-

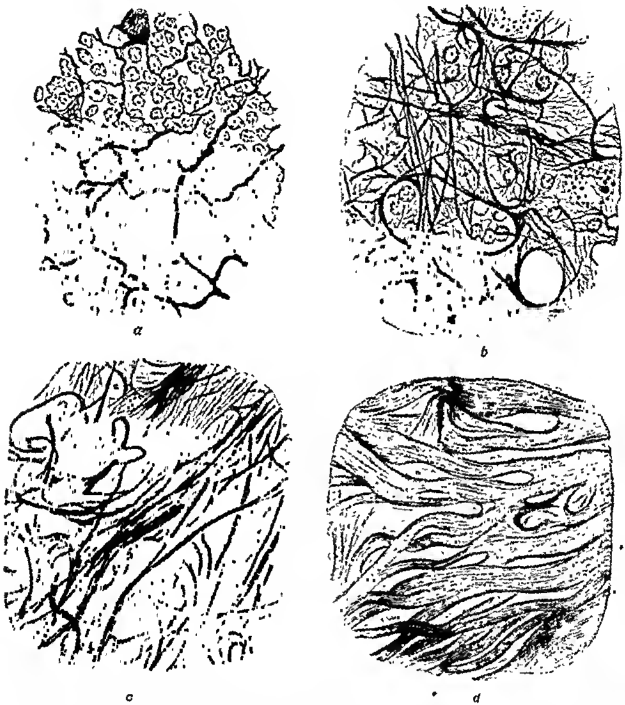

Fig. 86 Four stages in the development in tissue culture of collagenous fibers from the reticular tissue of a rabbit lymph node: *a*, Section of normal lymph node showing cells and reticular fibers (black); *b*, the reticular fibers are branching and much more numerous after four days in vitro; *c*, after five days in vitro the reticular fibers (black) are in sharp contrast to the newly formed collagenous fibers (gray); *d*, after six days in culture there are only thick bundles of collagenous fibers. Bielschowsky-Foot and Mallory-Azan stains. About 500 ×. After McKinney.

tinue into the argyrophil networks. The bundles of fibrils increase in thickness and finally lose the ability to be impregnated with silver (Fig. 86). Instead, they begin to stain with the methods for collagenous fibers (Mallory, von Gieson) and, like them resist digestion by pancreatin in an alkaline medium.

Stearns (1940), studying the development of fibers in transparent chambers in the ears of rabbits, concluded that the fibers develop extracellularly; she saw the fibroblasts give off parts of their cytoplasm which apparently were utilized in the production of the fibers.

The formation of the intercellular connective tissue fibers may be a gelation of a colloidal sol secreted by the cells. The final arrangement and direction of the collagenous bundles are probably influenced by mechanical forces.

Connective tissue fibers do not develop in scorbutic guinea pigs and they appear rapidly when such animals are given vitamin C. Phosphatase is present in large amounts in the developing collagen of a scar.

The *elastic* fibers appear in the embryo much later. Their histogenesis has not been adequately studied, but it is highly probable that the facts found for the collagenous fibers will be equally applicable to them.

POTENCIES OF DEVELOPMENT OF THE LYMPHOID STEM CELLS OF THE LYMPHATIC AND MYELOID TISSUES

Nearly all hematologists agree that the various myeloid elements of the bone marrow develop through proliferation and differentiation from a basophil free stem cell—designated here as the hemocytoblast. In the lymphatic tissue the small lymphocytes develop from young forms of larger size which have exactly the same structure as the hemocytoblasts in the bone marrow. *The question arises whether the lymphoid cells in both tissues have identical or different developmental potencies.* If they are identical, then all blood elements of the adult originate from one common stem cell which may appropriately be called the hemocytoblast (the "unitarian theory" of hemopoiesis). If, on the contrary, the large lymphoid cells of the lymphatic and of the myeloid tissues differ in their potencies, then each of these two tissues has a specific stem cell and one of these could be called the *lymphoblast*, the other the *myeloblast* ("dualistic" theory of hemopoiesis). The "trialistic" theory holds that the monocytes, too, have a distinct stem cell. There are many variations of each of these theories.

Much confusion is due to the fact that many hematologic theories rest almost exclusively on studies of the peripheral blood of man in health and disease. The unitarian theory, however, is based on a comprehensive comparative and histogenetic study of the blood and connective tissues in both embryonic and adult animals, and to a large extent on the results obtained from experimental studies on extramedullary myelopoiesis, inflammation, and tissue culture. In our opinion the sum of all of the facts speaks more in favor of the unitarian than of any of the pluralistic theories. In its light, the genetic interrelationships of the blood cells are much clarified and it becomes unnecessary to subdivide them into numerous cell lineages on the basis of minute, inconstant, structural differences (Fig. 87). In fact, the stem cells which appear in the blood in leukemia are now called "blasts" by most clinical hematologists until changes in the blood picture or the course of the disease permit the classification into myeloid or lymphatic type.

In the mammalian embryo both lymphoid and myeloid elements arise from the same lymphoid wandering cell.

In the lower vertebrates the boundary between myeloid and lymphoid is completely effaced and

ods give a clear insight into the morphology of this process. Delicate networks of fine, branching and anastomosing argyrophil fibrils appear on the surface and between the fibroblasts (Fig. 85). The fibrils may follow the outlines of the cell bodies and their processes, but they also extend far into the intercellular substance (nutritive medium in the case of the cultures). The first networks increase in number and thickness, and then are rearranged into parallel, wavy bundles which con-

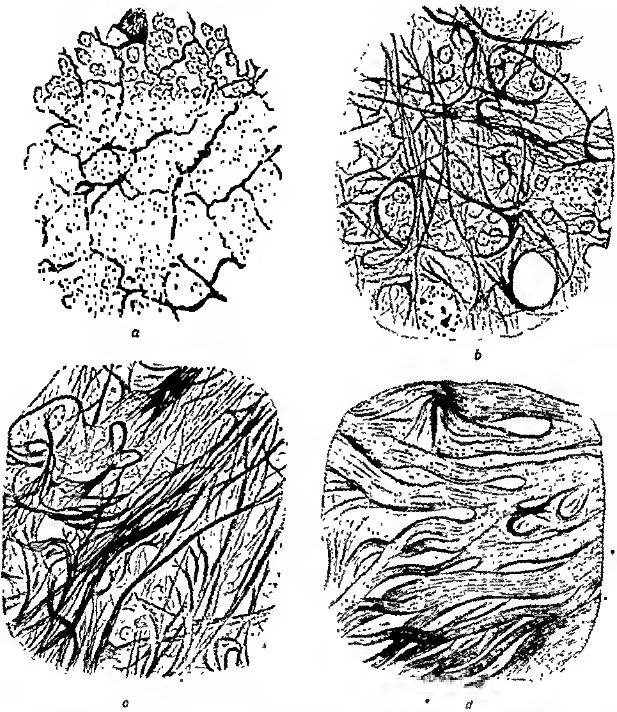

Fig. 86. Four stages in the development in tissue culture of collagenous fibers from the reticular tissue of a rabbit lymph node: *a*, Section of normal lymph node showing cells and reticular fibers (black); *b*, the reticular fibers are branching and much more numerous after four days in vitro; *c*, after five days in vitro the reticular fibers (black) are in sharp contrast to the newly formed collagenous fibers (gray); *d*, after six days in culture there are only thick bundles of collagenous fibers. Bielschowsky-Foot and Mallory-Azan stains. About 500 ×. After McKinney.

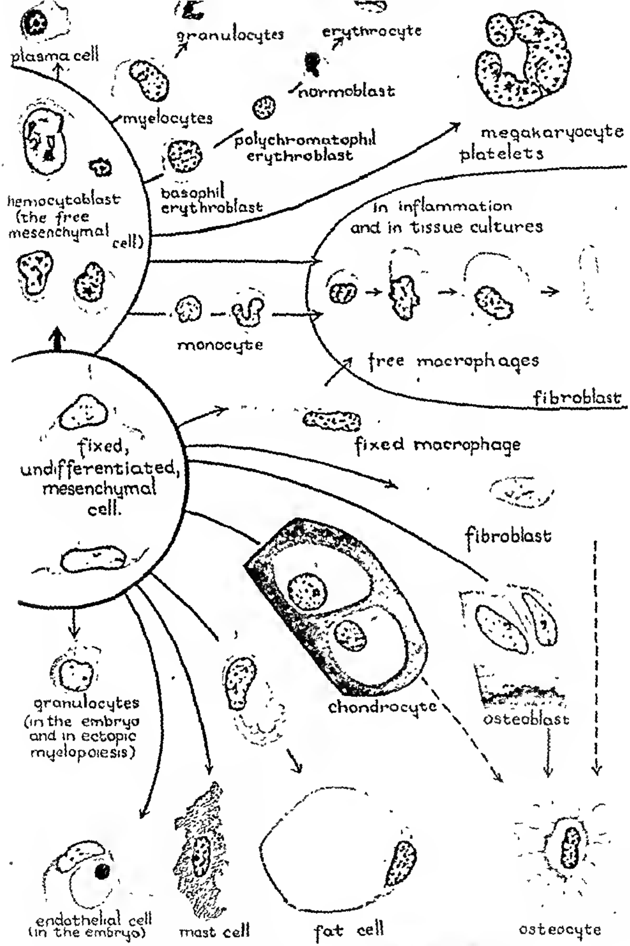

Fig. 87 The interrelationships of the cells of the blood and connective tissues of mammals. All of the cells are from human tissues. The dotted lines indicate unusual transformations. The lymphocytes are included with the hemocytoblasts. Hematoxylin-eosin-azure II. 720 ×. (W. B.)

developing lymphoid and myeloid cells are everywhere mixed with each other.

In certain circumstances the lymphocytes of the circulating blood may be transformed into myeloid cells. If a small fragment of a lymph node of an adult rabbit be cultivated in a medium containing bone marrow extract, the large lymphocytes (lymphoblasts or hemocytoblasts) may differentiate into myelocytes. Lymphocytes of the thoracic duct of ascaris immunized rabbits have also been observed to develop into heterophil leukocytes in cultures containing bone marrow extract and ascaris extract (Fig. 91). It has also been claimed that the monocytes of monocytic leukemia developed into myelocytes in tissue culture.

The myeloblasts of the blood in myeloblastic leukemia develop into macrophages and fibroblasts in tissue culture, just as do the lymphocytes of lymphatic leukemia under the same conditions. The dogma that myeloblasts can produce only *myelocytes and erythroblasts, is thus* refuted. These experiments also refute the so-called "trialistic theories" of blood formation, for in all of these cultures, both "myeloblasts" and "lymphocytes" pass through a monocyte stage before becoming macrophages.

It is generally admitted at present that structural differences between lymphoblasts and myeloblasts do not exist constantly. Biochemical and functional differences, as the presence of oxidizing and proteolytic enzymes in the myeloid elements and their absence in the lymphoid elements, were thought for a time to differentiate these two cell types; but these tests have been found wanting, because under certain circumstances the lymphoid cells also seem to elaborate these enzymes, while in other conditions the myeloid cells may lose them. Moreover, the idea that the two tissues develop independently in the embryo, is entirely unfounded DeBruyn showed that lymphocytes and myeloblasts have the same type of motion in culture.

Finally, the different reactions of myeloid and lymphatic tissues and especially of their stem cells in various diseases, with an apparent antagonism between them, seemed to argue in favor of the dualistic theory. In lymphatic leukemia, only the lymphatic tissue all over the body seems to be affected while the myeloid tissue atrophies. In myeloid leukemia, on the contrary, the myeloid elements flood the organism while the lymphoid tissue becomes scarce and even disappears. Experimental investigations have shown, however, that this different reaction is probably not due *to fundamental* differences in the tissues and their elements, but to *differences in the stimuli* in the two diseases. The stimulus of unknown nature which plays the decisive rôle in myelosis causes the common stem cell, the hemocytoblast, to differentiate in the myeloid direction, while the causative factor of lymphadenosis leads to a diffuse proliferation of the hemocytoblasts without their further differentiation.

Origin of the Monocyte. The questions of the origin and nature of the monocyte are among the most debated problems in morphologic hematology. According to various investigators, monocytes arise: (1) in the myeloid tissue, or (2) from endothelium, or (3) from macrophages, or (4) from lymphocytes (hemocytoblasts). The majority of the facts seem to support the idea that monocytes are lymphocytes, that is, hemocytoblasts, which have developed somewhat in the phagocytic direction. The main site of this transformation is the blood in the venous sinusoids of the spleen, liver, and bone marrow where *transitional* forms between lymphocytes and monocytes are common, especially in experimentally produced monocytoses (Fig. 39). In certain infections large numbers of monocytes develop in the lymph nodes (Conway, 1938).

Some believe the monocyte to develop from specific monoblasts in the bone marrow, but the morphologic specificity of the precursors has not been proved. Myeloblasts from the blood in myeloblastic leukemia pass through monocyte-like stages as they develop into macrophages in tissue culture. Most of the recent investigations indicate that endothelium does not furnish ameboid cells in the adult mammal.

The monocytes of the blood do not store vital dyes, as do the macrophages, both large and small It is clear, however, that the monocytes as they develop into macrophages soon take on the ability to store vital dyes. While it is theoretically possible that monocytes may develop directly from the fixed undifferentiated connective tissue cells of the blood forming organs, such a process has never been demonstrated

The unitarian hematologists consider the lymphocytes to be the source of monocytes. They point out that in blood smears of most animals it is impossible to separate all of the monocytes as a cell type distinct from lymphocytes. In the rat, and to some extent the monkey, supravital

phages. (3) Free cells which circulate in the blood or are scattered throughout the connective tissue. These are the *hemocytes;* among them are to be distinguished: (*a*) the hemocytoblasts (lymphocytes) which serve as stem cells for (*b*) granulocytes, monocytes, erythrocytes, and megakaryocytes.

The relations between all these cells are not clearly observable under physiologic conditions. Under pathologic or experimental conditions, when there is an increased destruction of cells and a corresponding intense new formation, the genetic relationships may be analyzed more easily. Three processes especially favor such analysis: (1) the changes in the tissue in the local "defense" reaction, that is, in inflammation, (2) the reactions of tissue in culture, and (3) extramedullary myelopoiesis.

It is clear from the facts detailed below that the various sized lymphocytes (hemocytoblasts) are all endowed with hemopoietic, phagocytic and fibrocytic potencies. The lymphocytes are free, mesenchymal cells which are scattered everywhere in the tissues of the adult body and circulate in the blood and lymph. Under normal conditions they keep the appearance of lymphocytes but, in response to certain pathologic stimuli, they may become granulocytes, erythroblasts, macrophages, etc. They may be looked upon as an easily movable mesenchymal reserve.

It has been shown that fixed cells with unrestricted mesenchymal potencies exist in the connective tissues of the adult mammals. According to various investigators these are fibroblasts or endothelial cells or fixed macrophages. But the most convincing evidence indicates that the original mesenchymal potencies are retained by cells scattered in the loose connective tissue along the blood vessels, by the primitive reticular cells of the hemopoietic tissues, and by some cells lining the venous sinuses of the liver.

In Inflammation. In inflamed connective tissue heterophil leukocytes migrate from the vessels at the very beginning of the process and rapidly degenerate in the tissue. The *mononuclear exudate cells* are ameboid, often phagocytic, nongranular cells with an oval or kidney-shaped nucleus. Because of the diversity of their appearance and their transformations they have been called *polyblasts.* According to various investigators they arise from: fibroblasts, endothelium, macrophages, blood lymphocytes or monocytes. The main rôle in their formation is played by the three last-named cell types.

In the course of the inflammatory process the fibroblasts proliferate and form the collagen of the scar of late stages. There is some evidence that they may develop into macrophages, but this transformation is denied by most investigators. In inflammation the endothelial cells of the common blood vessels are often found swollen and dividing mitotically. But they do not turn into free cells of any kind and do not produce ameboid or granular cells. The fixed macrophages, on the contrary, furnish a part of the polyblasts This is especially clear in vitally stained animals.

Although some of the polyblasts in all inflammatory lesions arise from the local fixed macrophages, a much more important source is the lymphocytes and monocytes of the blood. These agranulocytes migrate from the blood vessels into the tissue where they rapidly hypertrophy into large phagocytic elements. In the first two days after the onset of inflammation they can still be distinguished from the polyblasts of local fixed macrophage origin by their smaller size. But they continue to grow until after two days the polyblasts of local and of hematogenous origin can no longer be distinguished. In a vitally stained animal the discrimination of the polyblasts from the two sources becomes even more difficult as the hypertrophied lymphocytes and monocytes very soon begin to store vital dyes.

In different types of inflammation the polyblasts may assume various characters. In tuberculous lesions some of them become changed into the epithelioid cells of the tubercle. The polyblasts may form, through fusion, giant cells of the foreign body or of the tuberculous type.

In the later stages of inflammation, when scar tissue is formed, the polyblasts remain scattered among the fibroblasts and settle down as fixed macrophages; in still later stages, some become fibroblasts. In this way small lymphocytes and monocytes of the blood develop into macrophages and fibroblasts.

In Tissue Cultures. In tissue cultures the

staining with neutral red and Janus green shows the monocytes to be connected with the common small lymphocytes by a complete series of transitional forms. Similar transition forms between lymphocytoid and monocytoid wandering cells are to be found in the loose connective tissue. In inflammation the hematogenous lymphocytes and monocytes rapidly become ameboid phagocytic elements (polyblasts). In this progressive development the lymphocytes pass through a transitory state in which they cannot be distinguished monoblast which is different from a lymphocyte (hemocytoblast).

GENETIC INTERRELATIONSHIPS AND POTENCIES OF THE CELLS OF THE BLOOD AND LYMPH, THE CONNECTIVE TISSUE AND ENDOTHELIUM

From a general histologic point of view, three large groups of cells can be distinguished in the connective tissue and the

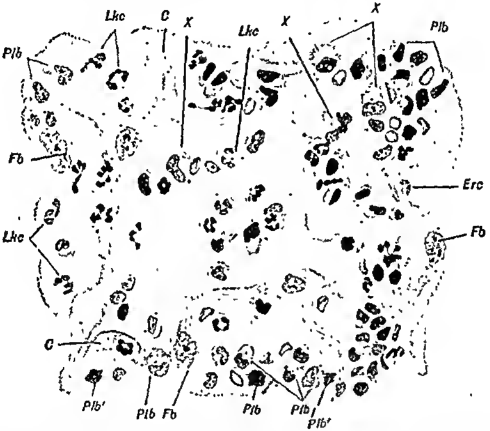

Fig. 88 Inflamed loose connective tissue of a rabbit; nineteen-hour stage. In the edematous tissue between the collagenous fibers (*C*) four cell types can be discriminated: hematogenous, heterophil leukocytes (*Lkc*); fibroblasts (*Fb*); hematogenous polyblasts arising from lymphocytes (*Plb'*) and from monocytes (*Plb*); mobilized fixed macrophages (histiocytes), *X*. The last two types of cells can still be sharply separated from each other; in later stages both types will form identical polyblasts; *Erc*, extravasated erythrocyte. Iron-hematoxylin stain. About 600 ×. (A.A.M.)

from monocytes and, among other characteristics, have a typical neutral red rosette. In cultures of normal and leukemic blood leukocytes, as well as of lymphocytes of rabbit lymph, the small lymphocytes change into monocytoid cells and then into large macrophages and finally into fibroblasts. In rabbits in which an extensive monocytosis has been produced, as by *B. monocytogenes*, the monocytes develop by individual hypertrophy from the common smaller lymphocytes. In none of this experimental material is there any evidence for the existence of a specific

blood: (1) Fixed, highly specialized elements. As fibroblasts they produce collagen; as endothelium, they line blood channels; and as chondrocytes and osteocytes, they form the cells of cartilage and bone. (2) Fixed or free cells which phagocytose, store vital dyes and other colloidal substances, and play important rôles in the general metabolism and especially in the "defense" reactions. These are the *macro-*

(2) In the lymphoid organs, including the spleen, the free basophil cells of the tissue, the lymphocytes, are often the source of the myeloid elements, this process has even been found in the germinal centers. (3) In still other cases the first myelocytes may develop directly from undifferentiated perivascular cells or primitive reticular cells with or without passing through a hemocytoblast stage.

Fig 90 Cells from sections of cultures of the leukocytes of the blood of a guinea pig, showing the development of lymphocytes and monocytes into macrophages (polyblasts) and the first stages of the change of the latter into fibroblasts All cells drawn at the same magnification—about 750 X. *a* and *b*, Lymphocytes and, *c*, monocyte from the freshly centrifuged blood, *d* and *e*, ameboid lymphocytes and, *f*, ameboid monocyte—three-hour culture; *g* to *k*, from a ten-hour culture; *l* to *n*, from a twenty-five-hour culture; *o* to *u*, from a two-day culture, *u* is a "fibroblast-like" cell; *v* to *z*, from a five-day culture, *x* is a "fibroblast-like" cell, *w* and *z* are in mitosis; *y* is a macrophage with ingested material; *aa* and *bb*, from twelve-day cultures; *aa* is still of macrophage type, while *bb* is turning into a fibroblast. Note mitochondria in all cells; *rbc*, erythrocyte. Iron-hematoxylin stain (A.A.M.)

Undifferentiated Fixed Cells. Primitive Reticular Cells. In the lymphatic and myeloid tissue the development of fixed and free macro-

fibroblasts proliferate faster than any other cells. Here, too, as in inflammation, they fail to change into other cell types.

In tissue cultures the lymphocytes and monocytes of the circulatory blood and the lymphocytes of the lymph develop into macrophages through a rapid transformation (Fig. 90). If the nutritive medium contains a vital dye, the hypertrophied lymphocytes and monocytes store it in granular form. In older cultures, the transformation proceeds further; the cells proliferate mitotically and turn into fibroblasts which form large sheets of connective tissue in which the development of argyrophil and collagenous fibers has been observed.

Extramedullary Myelopoiesis. Under physiologic conditions in adult mammals, and especially in man, the formation of the myeloid elements is confined to the bone marrow. In various abnormal conditions *extramedullary* or *ectopic myelopoiesis* or *myeloid metaplasia* is of common occurrence.

Local myelopoiesis has been observed in the sclerotic aortic wall, in the adrenal, and in other places; it develops after ligation of the renal artery and vein of the adult rabbit. More generalized extramedullary myelopoiesis occurs in various parts of the body in leukemia and certain intoxications and infections. It can be produced through repeated bleeding or chronic poisoning with blood-destroying substances. As a rule the spleen is the first organ affected. Later the liver, the lymph nodes, the adrenal and other organs become involved. Nearly always, it is the heterophil and eosinophil myelocytes which first appear in the new place; megakaryocytes come later and finally erythroblasts develop.

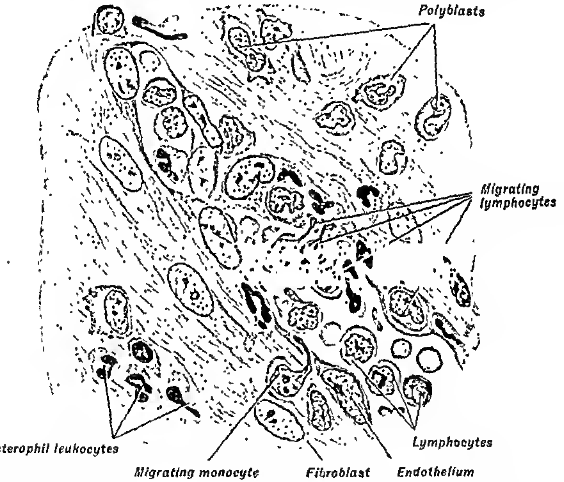

Fig. 89. A portion of the periphery of an area of inflammation in the subcutaneous tissue of a rabbit caused by the injection thirty-six hours previously of *Bacterium monocytogenes*. Hematoxylin-eosin-azure stain. 800 ×. After Bloom.

Experimental investigations have shown that the myelocytes and erythroblasts in extramedullary myelopoiesis originate in several different ways: (1) Sometimes the first myeloid elements appear in the lumen of the venous capillaries where they originate from lymphoid cells which circulate in the blood. From the viewpoint of the unitarian theory the latter are identical with lymphocytes. The newly formed myeloid elements may pass through the wall of the vessels into the tissue where they continue to proliferate.

(phagocytic reticular cell) can cease its phagocytic activity and become a primitive reticular cell.

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phages from the primitive reticular cells can be observed in the body and in cultures of these tissues. Lymphoid cells, hemocytoblasts, also originate from the same source, particularly in the germinal centers. In the omentum the new formation of fixed macrophages from perivascular, undifferentiated cells has been described. In extramedullary myelopoiesis the myelocytes can often be traced directly to perivascular, fixed cells. If foreign substances, especially foreign that under the influence of external stimuli, all fibroblasts can produce any other cell type of the blood and connective tissue. This has not been confirmed, however.

According to another opinion, macrophages of every kind are endowed with full mesenchymal potencies.

But the histogenesis of the lymphocytes of the lymphatic tissue shows that the lymphocytes arise not from the fixed macrophages, but from

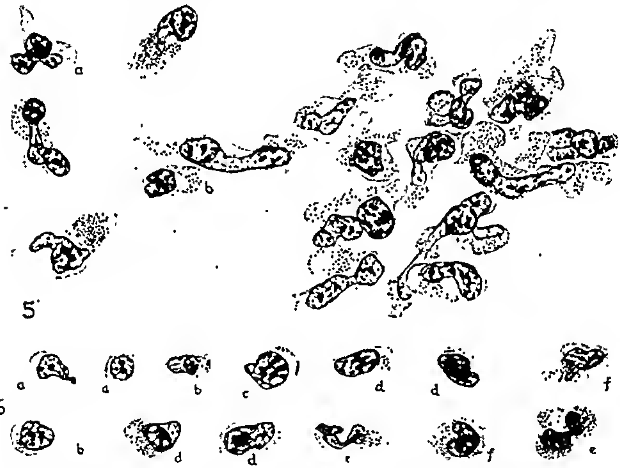

Fig. 91. Heterophil myelocytes and leukocytes which have developed from lymphocytes in tissue culture. The thoracic duct lymph of a rabbit immunized to ascaris extract was cultured with connective tissue in plasma and bone marrow and ascaris extracts. Cell *5a* is an unchanged lymphocyte. *5b* is an early myelocyte with a lymphocytic nucleus. The cells of *6* show stages in the development of lymphocytes *a*, *c* into myelocytes *b*, *d*, *e*, *f*. Hematoxylin-eosin-azure II. 1490 ×. Drawn by Miss E. Bohlman. After Bloom, 1937. Courtesy of Wistar Press.

proteins, are introduced into the organism, the macrophage system which has to dispose of them shows an increase in the size and number of its cells all over the body. These newly formed macrophages also have their source in the undifferentiated mesenchymal elements (primitive reticular cells).

The question as to which cells of the connective tissue represent these undifferentiated elements is answered in different ways by various authors. Some investigators express the opinion the primitive reticular cells which are also the source of the fixed macrophages. The same is true for the development of myelocytes in extramedullary myelopoiesis in the lymph nodes. The potencies of the fixed macrophages are undoubtedly greater than those of the fibroblasts. They can be transformed into phagocytic polyblasts, or giant cells, or fibroblasts. It has not been demonstrated that they give rise to monocytes, leukocytes, or red blood cells. The question is undecided whether a fixed macrophage

Chapter VI

CARTILAGE

In cartilage the intercellular substance forms a solid mass which permits this tissue, even when fresh, to be sectioned into thin films. The cells lie in special cavities of the interstitial substance. Because of differences in the interstitial substance, several types of cartilage may be distinguished, of which the most important are the hyaline, elastic, and fibrous varieties.

HYALINE OR GLASSLIKE CARTILAGE

This variety of cartilage is the most widespread and the most typical; the other types are modifications of it. In adult mammals it is found on the ventral ends of the ribs, on the surfaces of bones within joints, and in the respiratory passages. It is much more widespread in the embryo, where it constitutes most of the temporary skeleton.

Hyaline cartilage is a very flexible and somewhat elastic, semitransparent mass with an opalescent bluish tint, similar to that of frosted glass. It never possesses blood vessels of its own, although vessels supplying other tissues occasionally pass through it. With the exception of its naked surfaces in joint cavities, cartilage is always covered externally by a firmly attached layer of dense connective tissue—the *perichondrium*.

Cells of Cartilage (Chondrocytes). The cells of hyaline cartilage usually are spherical, although there are many exceptions Thus, in the layers of the cartilage under the perichondrium or under the free joint surface, the cells are flattened in a plane parallel with the surface and, in the lower layers, they are hemispherical or angular because of mutual pressure. On the border between the cartilage and the perichondrium there are intermediate forms between the cartilage cells and ordinary fibroblasts.

F ... Hyaline cartilage from the xiphoid process of a rat: *a*, Transition layer adjacent to perichondrium; *b*, continuation of collagenous fibers from the perichondrium into the interstitial substance of the cartilage; *c*, columns of isogenous groups of cartilage cells, some of which have fallen out of the cavities. Eosin-azure stain. 750 ×. (A.A.M.)

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The amorphous character of the interstitial substance is only apparent. It is thoroughly penetrated by thin collagenous fibrils which either form a dense feltwork running in all directions, or gather into definitely oriented bundles (Fig. 96). The interstitial substance seems to be homogeneous in the fresh state and after ordinary fixation, because the collagenous fibrils are covered with a binding mass of the same index of refraction. The collagenous fibers can be demonstrated with the silver impregnation methods or by digesting the tissue with trypsin which does not affect the fibers (see p. 57). The

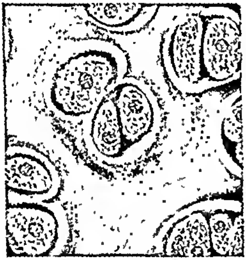

Fig. 94. Hyaline cartilage of a calf. Redrawn and modified after R. Krause. 400 ×.

collagen from cartilage seems to be much the same chemically as that from the loose connective tissue.

The interstitial substance of hyaline cartilage is markedly basophil due to its content of *chondromucoid*, a complex protein which yields *chondroitic acid* on hydrolysis. The intercellular substance immediately surrounding the capsules is often especially basophil; this is believed to be due to a concentration of chondromucoid in these areas.

Cartilage is devoid of blood vessels; as it forms large, compact masses and, as in the higher vertebrates, the great majority of cartilage cells have no processes and lie in completely isolated capsules, the nutritive fluid from the blood vessels in the perichondrium must pass through the interstitial substance to reach the cells.

Many authors have tried to prove the existence of a system of liquid conducting canaliculi pass-

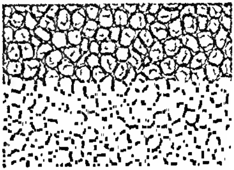

Fig. 95. Cellular cartilage containing very little ground substance, from the ear of a mouse. Dehydrated and mounted in damar. 100 ×. After Schaffer.

ing from one cell capsule to another through the *interstitial substance* of the cartilage. Systems of fibrils connecting neighboring cell groups have been described by other authors. But at present these structures are considered to be artefacts. Those reagents which extract

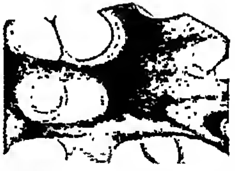

Fig. 96. Portion of a tracheal cartilage of a guinea pig from which all constituents except the collagenous fibers have been removed by digestion with trypsin. Redrawn after Rupprichit.

much water cause a wrinkling of the interstitial substance which may simulate structures. Direct observation of the action of a nontoxic stain upon living cartilage shows that the dye is quickly and evenly absorbed by the interstitial substance. In probably the same manner, the interstitial substance of the cartilage is permeated in the living condition by the tissue fluids from the perichondrium.

The body of the cartilage cell completely fills the cavity which it occupies in the interstitial substance. In the adult, higher vertebrates, the cartilage cells rarely have any processes and, as they are not connected with the wall of the cavity, they may drop out if the cavity is opened. Occasionally in the cartilage of the joints of the higher vertebrates and frequently in lower vertebrates and in early embryonic stages, the cytoplasm may extend into processes which enter the interstitial tissue (Fig. 93); the cells correspond in this respect with those of the ordinary connective tissue.

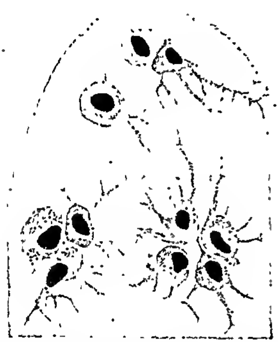

Fig. 93. Groups of cells with long, branching, anastomosing processes in the homogeneous interstitial substance of a cartilage of *Sepia officinalis*. 330 ×. (A.A.M.)

The cytoplasm of the cartilage cells contains long mitochondria, vacuoles, fat droplets, and glycogen. The vacuoles in the peripheral portion of the cell body frequently are so large as to distend the cell like a bubble. During fixation such cartilage cells become wrinkled very easily. Around the nucleus one can sometimes distinguish a cytocentrum with centrioles and a Golgi net. The nucleus contains one or several spherical nucleoli.

Mitotic figures are probably never found in the cartilage cells in the mature organism. In the cartilage of the adult, the cells are usually gathered into large or small, compact groups (Fig. 94). These cell groups may be rounded or they may be stretched into small columns of flattened cells lying in one line. Inside of the groups, between the separate cells, the interstitial substance may be compressed into thin bands or may even be absent. This distribution of the cartilage cells is the result of their multiplication during the last phases of development. After they are surrounded by a solid interstitial substance, they divide several times in rapid succession. In this way, individual cartilage cells give rise to groups which remain close together, imprisoned in the intercellular matrix. As these groups arise by division of one cell they are called *isogenous*.

Interstitial Substance. When the cells are closely packed, the interstitial substance of hyaline cartilage appears as a framework of thin cross beams surrounding the cartilage cavities. But more often it appears as a solid mass in which separate cells and groups of cells are dispersed at some distance from one another. In fresh condition and after ordinary fixation, the substance seems to be quite homogeneous. However, the layers adjacent to the cell cavities and forming their walls are always distinguishable by being more refractile, by their somewhat different staining reactions and, sometimes, by their concentric striation (Fig. 94). They seem to be envelopes of the cartilage cells and are called *cartilage capsules*.

Although the capsules are considered by many to be a transformed, peripheral layer of the cytoplasm, they undoubtedly belong to the intercellular substance. The capsules are not sharply marked off from the interstitial material; they are merely the layers of it which are the youngest and nearest to the cells.

ELASTIC CARTILAGE

In mammals this variety of cartilage tissue is found in the external ear, the walls of the external auditory and eustachian tubes, the epiglottis, and in parts of the corniculate and cuneiform cartilages. It differs from the hyaline cartilage macroscopically, by its yellowish color and by its greater opacity, flexibility, and elasticity.

Its cells are similar to those of hyaline cartilage; they are of the same rounded shape, are also surrounded by capsules, and are scattered singly or in isogenous groups of two or three cells. The interstitial substance differs from that of hyaline cartilage by being penetrated in all directions by frequently branching fibers, which give all of the tests for elastin (Fig. 97). They form a network which is often so dense that the amorphous substance filling its loops is obscured; sometimes the network becomes distinctly denser at the periphery of the cells. In the layers beneath the perichondrium, the feltwork of the elastic fibers is less dense. The elastic fibers of the cartilage continue into those of the perichondrium.

Here, as well as in hyaline cartilage, the phenomena of calcification and asbestos transformation may take place with advancing age.

FIBROCARTILAGE

Fibrocartilage occurs as indistinctly outlined, small accumulations in a few places in the bodies of mammals. It is found in the intervertebral disks, certain articular cartilages, in the symphysis pubis, in the ligamentum teres femoris, in the places of attachment of certain tendons to bones, etc. Here again, the tissue contains typical, spherical or oval cartilage cells with homogeneous capsules which lie either singly or in pairs, and sometimes in larger or smaller groups extended lengthwise. The interstitial substance contains thick, compact, collagenous bundles, parallel with one another and separated only by narrow clefts into which are squeezed the capsules containing the cells (Fig. 98). In typical cases very little is seen of the amorphous interstitial substance.

Fibrocartilage is closely associated with the dense connective tissue of the capsules

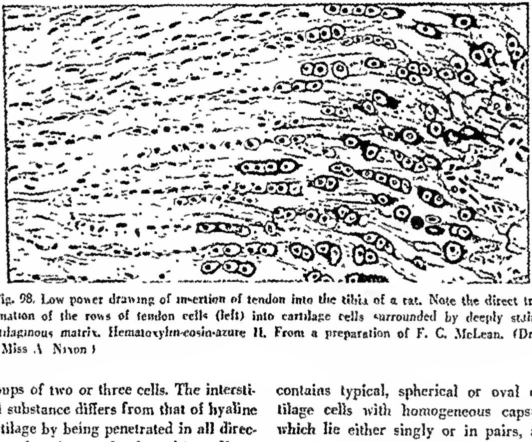

Fig. 98. Low power drawing of insertion of tendon into the tibia of a rat. Note the direct transformation of the rows of tendon cells (left) into cartilage cells surrounded by deeply staining cartilaginous matrix. Hematoxylin-eosin-azure II. From a preparation of F. C. McLean. (Drawn by Miss A. Nixon.)

Regeneration of Cartilage. After a wound or excision of a portion of living hyaline cartilage in adult mammals, an independent regeneration of the cartilage does not take place according to most observers. In the injured area only necrotic and atrophic changes can be observed in the cells. The defect is quickly filled by newly formed connective tissue, which grows in from the perichondrium or nearest fascia. Then the fibroblasts of this granulation tissue become round, produce capsules around themselves and may become transformed into new cartilage cells. Meanwhile, the fibrillar, interstitial substance of the scar tissue becomes homogeneous and gives rise to new interstitial substance in the manner described below for the embryonic development of cartilage tissue. Accordingly, in adult organisms, new cartilage tissue is formed by metaplasia of the loose connective tissue.

Such a metaplasia sometimes takes place in connective tissue under the influence of simple mechanical forces acting from the outside, such as pressure, particularly when combined with friction, etc. It is claimed that the presence of cartilage on the joint surfaces of the bones is connected with the constant mechanical influences to which a normal joint is subjected during its function. When these mechanical conditions disappear, as happens in dislocation of bones, the cartilage often undergoes dedifferentiation. On the other hand, cartilage is laid down in the primordia of the joint surfaces in the embryo at a time when there are probably no mechanical forces acting on the joints. Some cartilage cells may develop into osteocytes (see p. 137).

Regressive Changes in Cartilage. *Calcification* is one of the normal regressive changes of cartilage. This process is characterized by a deposition of minute granules of calcium salts in the interstitial substance, primarily in the vicinity of the cells. The cartilage becomes opaque, very hard and brittle. Calcification usually precedes the replacement of cartilage by bone. In man, ossification may take place in certain cartilages of the larynx as early as twenty years of age

Hyaline cartilage may undergo the so-called *asbestos transformation.* Within the homogeneous intercellular substance, parallel fibers are deposited which have nothing in common with collagenous fibers. They do not swell in acetic acid, but dissolve in boiling water and in low concentrations of alkalis. They give the tissue a silky, glossy appearance similar to asbestos; they spread over wide areas and may lead to a softening of the tissue and even to the formation of spaces in it. It has been reported that new cartilage may develop in these spaces.

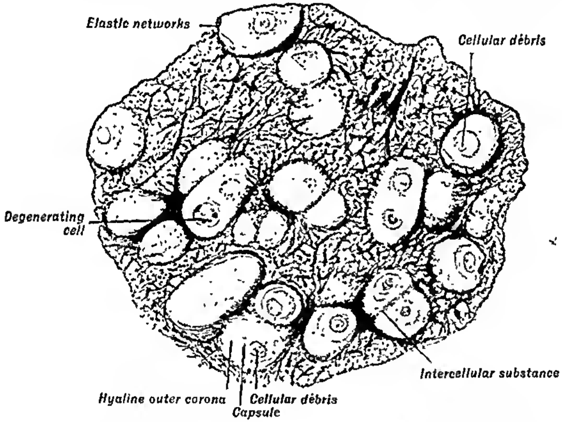

Fig. 97 Cartilage of the human ear. Orcein stain. 380 ×. After Schaffer.

cells. Some cells atrophy, become compressed between neighboring cells, and eventually disappear.

With the gradual increase of the interstitial substance, there appears a very thin, progressively thickening, shining layer—the capsule—along the lines of its contact with the cytoplasm of the cartilage cells. This structure develops in the same manner as the rest of the interstitial tissue, of which it represents the youngest layers.

The above described multiplication of the cells by mitosis and the increase in mass of the intercellular substance is called *interstitial growth*.

The mesenchyme surrounding the cartilage primordium forms a special layer, the *perichondrium*, which merges gradually with the cartilage on one side and the adjacent embryonic connective tissue on the other. Throughout embryonic life there is a constant transformation of layers of this connective tissue into cartilage. Here the acidophil collagenous fibers of the dense connective tissue of the perichondrium are arranged in flat bundles; these are gradually covered with the basophil, cartilaginous, ground substance. At the same time the fibroblasts of the connective tissue lose their spindle shape, change into spherical cells, and thus are transformed directly into cartilage cells surrounded by capsules. This process is called *appositional growth*; it probably contributes more to the mass of the cartilage than does the interstitial growth. The ability of the perichondrium to form cartilage persists, although latent, in the adult organism.

Most of the hyaline cartilage of the embryonic skeleton is later replaced by bone (Chapter VII). Some cartilages are completely absorbed, as is the case with Meckel's cartilage.

At the site of the future elastic cartilage in the embryo, there is at first connective tissue containing fibroblasts and wavy fibrillar bundles which do not give characteristic reactions for either collagen or elastin. These indifferent fibers apparently are partly transformed into the elastic fibers. The cells which are surrounded by capsules become cartilage elements. On the surface of the elastic cartilage there is also a perichondrium which serves during embryonic life for the appositional growth of the cartilage.

The development of the fibrocartilage differs little from the development of ordinary connective tissue. In the beginning there are ordinary fibroblasts separated by a large amount of fibrillar substance. Then the cells become round, are surrounded by capsules, and are directly transformed into cartilage cells. The interstitial substance becomes infiltrated only slightly if at all with the basophil, amorphous, binding mass.

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and ligaments of joints. It is a transitional form between cartilage and connective tissue, and this gradual transformation can always be observed, in the embryonic histogenesis as well as in the adult organism, wherever there is fibrocartilage. Thus, in the intervertebral disks, the hyaline cartilage connected with the vertebrae

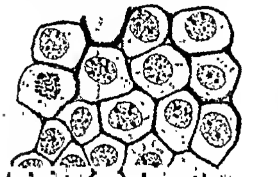

Fig. 99. Development of cartilage from mesenchyme in a 15-mm. guinea pig embryo. The mesenchymal syncytium (below) gradually merges into the protochondral tissue with interstitial substance (above). Note mitoses 750 ×. (A.A.M.)

at first shows distinct collagenous fibers in the apparently homogeneous interstitial substance. Then these fibers collect into thick bundles which almost entirely displace the homogeneous substance, while the cartilage cells retain their spherical form and their capsules. Finally, this typical fibrocartilage goes over into connective tissue, the cells of which are provided with processes and are devoid of capsules.

Other Varieties of Cartilage and Chondroid Tissue. There is a transitory phase in the embryonic development of the hyaline cartilage when it is composed of closely adjacent, separate, vesicular cells, provided with thin, but very stable elastic capsules, and with collagenous fibers in its interstitial substance. In this undeveloped condition, the cartilage may remain throughout life in certain places in the body of higher organisms. It occurs frequently in lower vertebrates (fishes, amphibians; as in the sesamoid cartilage of the tendon of Achilles in frogs) and is still more frequently observed in invertebrates. Such tissue has been called by several names—*pseudocartilage, fibrohyaline tissue, vesicular supporting tissue, chondroid tissue,* etc. This cartilage-like tissue serves as a mechanical support to other parts of the body.

The tissue composing the notochord of vertebrates possesses a quite similar structure. Here, there is a shaft of variable thickness which consists of large, closely packed, vesicular cells distended with fluid and with an elastic membrane. The notochordal tissue has a different embryologic origin from that of the cartilage and of the other connective tissues (p. 28).

Histogenesis of Cartilage. In those parts of the embryo where cartilage will develop, the mesenchyme cells round up and the spaces between them become smaller. In most cases, at very early stages in the formation of cartilage, collagenous fibrils are present in the intercellular substance. This material, at first acidophil, soon develops into the distinctly basophil homogeneous intercellular substance characteristic of cartilage (and stains metachromatically with certain basic aniline dyes; for example, purple with methylene azure). Although most authors consider this substance to be transformed "ectoplasm" of the chondroblasts, it is probably a secretion of the cells (p. 105). In any event, it masks the collagenous fibrils embedded in it.

The cells enclosed by the interstitial substance quickly acquire the characteristic distinctiveness of cartilage cells. They accumulate large amounts of fluid in vacuoles in their cytoplasm and become spherical or, as a result of mutual compression, polyhedral. Mitoses may be observed among them for a long period; during the constriction of the cytoplasm in such a division, a new partition of interstitial substance quickly develops between and separates the two daughter

connective tissue, called *periosteum;* a somewhat similar tissue, the *endosteum,* lines the marrow spaces, including those in spongy bone.

Bone develops through a transformation of connective tissue (*intramembranous ossification*) or by a replacement of cartilage (*intracartilaginous* or *endochondral ossification*) or through a combination of these processes. The formation of bone tissue takes place by apposition, new bone being laid down upon connective tissue, upon cartilage matrix, or upon bone itself. As a bone grows in size it undergoes *internal reconstruction,* which continues throughout the life of the animal, although at a greatly reduced rate in adults. As a result of the reconstruction during growth, mature bone acquires a very complex structure.

Microscopically, by far the greater part of the mass of bone is made up of layers (*lamellae*) of calcified *interstitial substance* or *bone matrix;* the arrangement of the lamellae differs in spongy and in compact bone. By appropriate methods the lamellae are found to be fibrillar in structure. Embedded within the interstitial substance are *lacunae* (cavities), completely filled with bone cells (*osteocytes*). In the walls of the lacunae are fine apertures, from which arise numerous thin canals, the *bone canalicules,* which penetrate the hard interstitial substance in all directions. They branch abundantly and anastomose with one another in a sort of network, connecting all the lacunae into a system of cavities. On the surfaces of bone, and much more numerous during the active development and growth of the skeleton, are *osteoblasts* and *osteoclasts,* associated respectively with the apposition and the reconstruction of bone. The contribution of the cellular elements of bone to its total mass is small.

The Cells of Bone. Osteoblasts, osteocytes and osteoclasts, peculiar to bone, are closely interrelated, in that transformations from one to another of the three cells are frequenly observed.

The *osteoblast,* associated with the formation of osseous tissue, appears on the surface of bone which is undergoing growth and development; these cells are frequently present in a continuous layer, suggesting a cuboidal epithelium (Fig. 101) and they are found in this location as long as active growth occurs. The body of the osteoblast has a diameter of 15 to 20 μ. The nucleus is large, and there is usually one fairly large nucleolus. The cytoplasm of the osteoblast stains intensely with basic anilin dyes, suggesting the presence of ribose nucleic acid. This, together with the presence of *phosphatase* in these cells, suggests that the cells are concerned with the synthesis of the proteins of the bone matrix. The cytoplasm also contains numerous, threadlike mitochondria; near the nucleus is a pale staining attraction sphere with a diplosome and a Golgi net. The osteoblasts are often connected with one another by thin cytoplasmic processes.

The *osteocyte* is frequently, perhaps usually, an osteoblast which has become embedded within the bone matrix (Fig. 100). It has a faintly basophil cytoplasm, containing a few mitochondria and a small Golgi net. It is uncertain whether it contains a cytocentrum and centrioles. When stained supravitally with neutral red, it contains neutral red vacuoles, especially in young bone. Fat droplets may occur; glycogen has not been demonstrated. The oval nucleus is large and filled with large chromatin particles and one or more nucleoli. In general, the appearance of the osteocytes suggests that of a somewhat shrunken fibroblast with a darker nucleus. Cells with two nuclei occur; mitoses have not been described in osteocytes.

The shape of the lacunae in which the osteocytes lie is usually flat and oval, resembling that of a melon seed. They range

Chapter VII

BONE

BONE is a hard, specialized connective tissue, with a calcified collagenous intercellular substance. It performs a mechanical function in forming the *skeletal support of the body;* it protects the vital organs of the cranial and thoracic cavities and lodges the bone marrow. Its structure, even to minute details, is beautifully adapted to the performance of its supporting function with the least expenditure of material and with the least weight. As a second important function, related to its impregnation with minerals, bone serves as a store for calcium, and thus plays a part in the meeting of the immediate needs of the organism for this element.

Despite its passive character, low metabolic rate, and great content of inorganic material, bone is a very plastic tissue and is highly sensitive to alterations of its normal mechanical function. Thus disuse is followed by *atrophy,* in this case associated with a loss of substance, while increased use is accompanied by *hypertrophy,* with an increase in the mass of the bone. Owing to the ability of the bone to undergo internal reconstruction in response to external stimuli, it may to some extent be modified at will by surgical and experimental procedures.

Macroscopically, mammalian bone is either *spongy* (*cancellous*) or *compact* in structure. Spongy bone consists of intercrossing and connecting osseous bars of varying thickness and shapes. These branch, unite with one another, and partially surround intercommunicating spaces filled with bone marrow, by their arrangement giving the skeleton a maximum rigidity and resistance to changes in shape. Compact bone appears as a continuous hard mass in which spaces can be distinguished only with the aid of the microscope. No sharp boundary can be drawn between the two types of bone tissue; they are merely different arrangements of the same histologic elements. Moreover, practically every bone contains both types of osseous tissue.

In typical long bones (femur and humerus) the *diaphysis* (shaft) consists of compact bone and contains in its center a voluminous, cylindrical, bone marrow cavity. The *epiphysis* (at the end of the shaft) consists of spongy bone with a thin, peripheral cortex of compact bone. The cavities of this spongy bone are, in the adult animal, direct continuations of the bone marrow cavity of the diaphysis. In the growing animal the epiphysis and diaphysis are separated by the *epiphyseal cartilage* plate, which is united with the diaphysis by columns of spongy bone, often called the *metaphysis.* The epiphyseal cartilage, together with the spongy bone of the metaphysis, form a *growth apparatus,* within which growth in length of the long bones occurs.

In the flat bones of the skull, the compact substance forms a relatively thick layer on both surfaces, between which there is a layer of spongy bone of varying thickness (*diploë*). The short and irregular bones usually are of spongy substance covered with a layer of compact bone.

All bones are covered with a modified

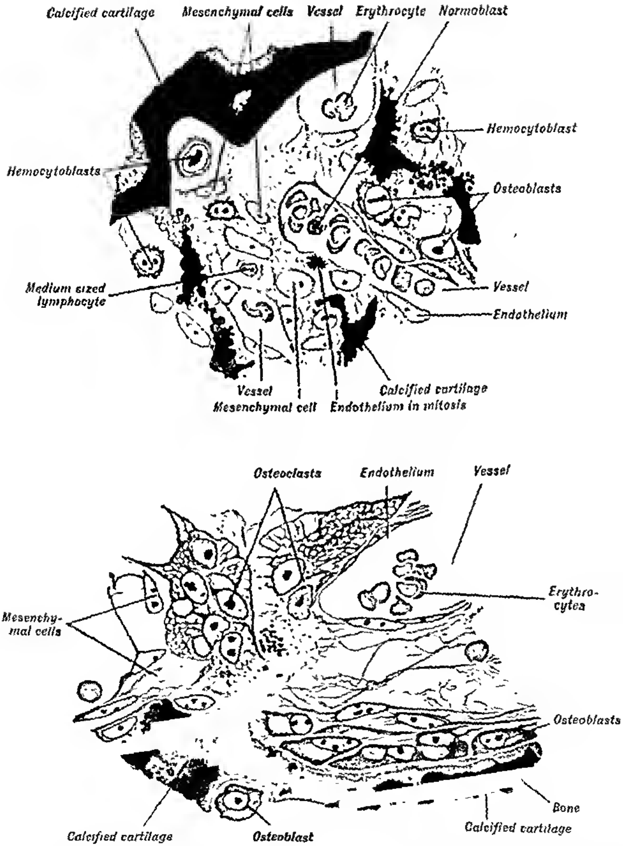

Fig. 101. Two areas from sections of the bone marrow cavity near the zone of endochondral ossification of the humerus of a human embryo of 70 mm. Hematoxylin-eosin-azure stain. About 700 ×. (A.A.M.)

in size from 22 to 52 μ in length, 6 to 14 μ in width, and 4 to 9 μ in thickness. On the surface of the cell bodies are many fine projections which enter the corresponding apertures in the walls of the lacunae. In the early stages of development of bone in mammals, thin cytoplasmic processes penetrate the bone canalicules so that the bone cells are directly connected. How far these cytoplasmic processes extend into the bone canalicules in adult mammals has not been determined.

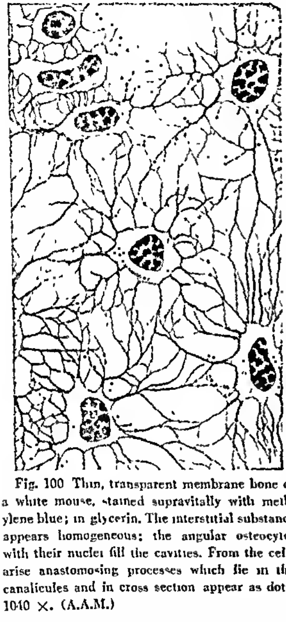

Fig. 100 Thin, transparent membrane bone of a white mouse, stained supravitally with methylene blue; in glycerin. The interstitial substance appears homogeneous; the angular osteocytes with their nuclei fill the cavities. From the cells arise anastomosing processes which lie in the canalicules and in cross section appear as dots. 1040 ×. (A.A.M.)

The *osteoclast* is a multinucleated giant cell, varying greatly in size and in the number of nuclei. These cells are derived from the stromal cells of the marrow; at times they arise by the fusion of a number of osteoblasts, and they may also include osteocytes liberated from bone by resorption. Their pale staining cytoplasm is often foamy; they frequently have branching processes with serrated edges (Fig. 101). The numerous nuclei are poor in chromatin and each has a prominent, although small, nucleolus.

The Interstitial Substance. The apparently homogeneous interstitial substance of fresh bone contains masked fibers, called *osteocollogenous fibers* (*ossein*), similar to the collagenous fibers of loose connective tissue. By silver impregnation and other special methods it can be shown that the individual fibrils are often connected into small bundles 3 to 5 μ thick; they are believed to be united by an amorphous binding substance. According to most investigators, it is in this organic binding substance that the mineral constituents of bone are laid down. In favor of this view is the fact that after igniting thin sections of bone, fine canalicules can be seen in the interstitial substance in the places formerly occupied by the fibrils.

A specialized, thin layer of the interstitial substance directly adjoins the lacunae and the canalicules and forms a sort of capsule for them. It differs from the rest of the interstitial mass in that it lacks fibrils, and by not dissolving when heated *in a solution of strong alkali.*

Chemical Composition of the Interstitial Substance. The hard interstitial substance is chemically similar in quite different types of animals. Besides water, the amount of which varies greatly and which is abundant in the bones of young animals, the interstitial substance consists of two main components: the organic framework, and inorganic salts. Of compact bone the inorganic part, or *bone ash,* chiefly the *bone salt,* attains a maximum of approximately 65 per cent of the

parts are usually arranged in those directions which correspond with the lines of maximum pressure or tension. The trabeculae of the spongy substance are made up of a varying number of closely adjoining bone plates, or lamellae. Embedded in the interstitial substance are the lacunae, containing osteocytes and intercommunicating with each other through a network of canalicules.

In the compact substance, the lamellae are regularly arranged, in a manner closely connected with the distribution of the blood vessels which nourish the bone. The compact substance of the diaphysis of any long bone is penetrated by numerous cylindrical, branching and anastomosing canals. These are the *Haversian canals;* they contain blood vessels with a small amount of connective tissue. They communicate by the *canals of Volkmann* with the external surface of the bone and with the bone-marrow cavity. It is by the Haversian canals and their related structures that the internal structure of compact bone differs from that of spongy bone.

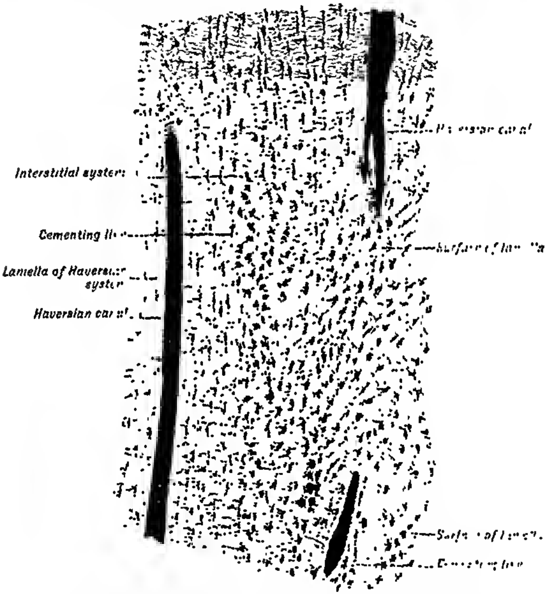

Fig. 103. From a longitudinal, *ground section* of the ulna of man; stained with fuchsin. 160 ×. (After Schaffer.)

The Haversian Systems. The Haversian system is the unit of structure of compact bone. It is an irregularly cylin-

dry, fat-free weight in adult life. In developing bone, especially in the embryo, this proportion is lower, and it may be as low as 30 to 35 per cent in the poorly calcified bone of *rickets* and *osteomalacia*. Neglecting the small admixture of other elements, the bone salt has the *empirical* formula $CaCO_3.nCa_3(PO_4)_2$, in which *n* has a value of approximately 2.5. Except for the well established fact that this salt gives the *x*-ray spectrogram characteristic of minerals of the *apatite* series, the exact details of the crystal structure are still in doubt. Considerable quantities of citrate, constituting the major portion of the body's stores of this salt, are found in bone; its relation to the bone salt is not known. Study of the turnover of the inorganic substances in bone is greatly aided by the use of radioactive isotopes (Fig. 102). Certain radioactive substances accumulate in bone, and may cause severe damage to the bone tissue.

The organic portion of compact bone, amounting to 35 per cent or more, is made up chiefly of *bone collagen* or *ossein*; only a small fraction of the dry weight is contributed by the bone cells. The organic framework yields gelatin when boiled.

A weak acid removes the inorganic salts, leaving the original appearance and microscopic structure. If a bone be ignited, only the inorganic constituents remain; a bone so treated becomes very brittle, although it retains its external form and, to a certain degree, its microscopic structure.

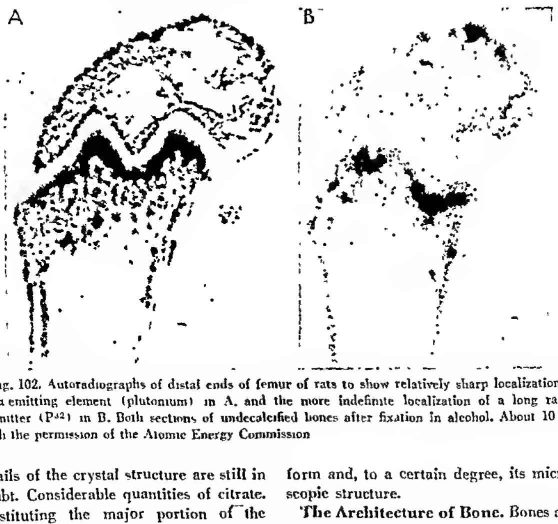

Fig. 102. Autoradiographs of distal ends of femur of rats to show relatively sharp localization of an α emitting element (plutonium) in A, and the more indefinite localization of a long range β emitter (P^{32}) in B. Both sections of undecalcified bones after fixation in alcohol. About 10 ×. With the permission of the Atomic Energy Commission

The Architecture of Bone. Bones are formed of two types of tissue, both found in nearly every bone. The cortex of a bone is commonly compact osseous tissue, while cancellous, or spongy bone is found in the medulla. Spongy *bone* is *simple in structure*, but *varied in form*. It consists of tubes, plates and bars, forming a network especially fitted for definite mechanical functions in individual bones; the

more, usually two, blood vessels. These are for the most part capillaries and post-capillary venules, lying in close association with the loose connective tissue which fills the remainder of the canal; occasionally an arteriole is found in a canal. The canalicules of the Haversian systems are extra-vascular, their function presumably being to promote the diffusion of the tissue fluids required for the maintenance of the osteocytes and of the interstitial substance of the bone. In a typical Haversian system (Fig. 104) canalicules branch out from the canal and form a network which includes the lacunae. The canalicules, for the most part, are directed radially, forming the channels of communication between the canal and the successive lamellae of the Haversian system and their lacunae, and circumferentially and longitudinally, providing for intercommunication between the

Fig. 105. Cross section of human hip bone in polarized light. The cross sections of three Haversian systems are seen as bright Maltese crosses. 130 ×. Redrawn after Gebhardt.

lacunae of the same lamella. The radially arranged canalicules communicate with the broad surfaces of the lacunae; the circumferential and longitudinal canalicules project from the thin edges of the lacunae. In the outermost lamella of an Haversian system the external canalicules loop back into the system; intercommuni-

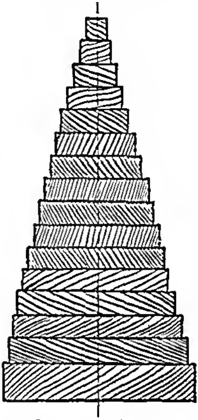

Fig. 106. Diagram of the direction of the fibrils in successive plates of an Haversian system. Redrawn and slightly modified from Gebhardt.

cation between the canalicules of adjacent systems is the exception rather than the rule.

In cross sections of an Haversian system, stained for connective tissue fibers, are seen lamellae which are alternately longitudinally and circularly fibrillated. The alternation of perfectly longitudinal and circular lamellae, however, occurs very rarely. More frequently this arrangement is only approximated and the fibrils in all the lamellae run spirally to the axis of the canal. These spirals in adjacent plates cross at various angles and are sometimes perpendicular to one another. The direction of the fibrils within the plates of an Haversian system is shown diagramatically in Fig. 106. The alterna-

drical, branching and anastomosing structure, with thick walls and a narrow lumen, the Haversian canal. The canals are generally from 22 to 110 μ in diameter; they are surrounded by concentrically arranged plates or lamellae of bone, of which there while in longitudinal sections the canals appear as long slits (Fig. 103). In addition to the canals and lamellae, the Haversian systems include large numbers of lacunae, each containing an osteocyte, and of canalicules, forming a network

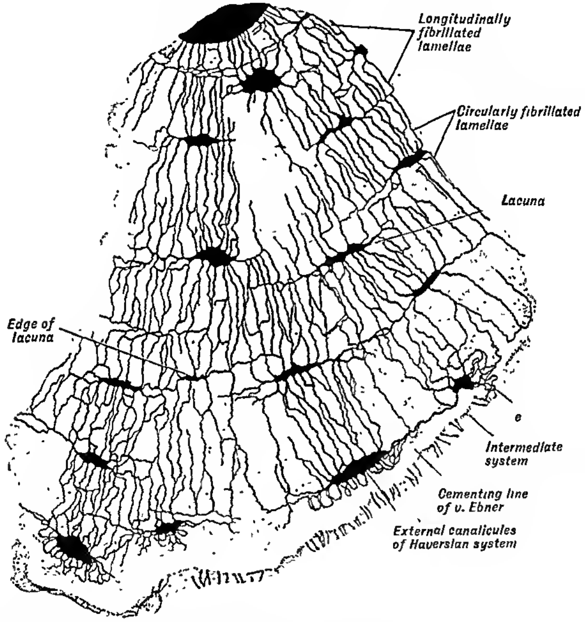

Fig. 104. Sector of a cross section of an Haversian system of a macerated human hip bone. The cavities and canalicules are filled with a dye: *e*, connection of canalicules of the Haversian system with those of an intermediate system. 520 ×. (A.A.M.)

may be from 4 to 20 in a single system, each from 3 to 7 μ in thickness. The Haversian systems are directed mainly in the long axis of the bone, so that in cross section the canals appear as round openings and the lamellae are ring shaped, branching out from the canals and intercommunicating with each other and with the lacunae. The broad surfaces of the lacunae are circumferentially placed in the lamellae.

An Haversian canal carries one or

more, usually two, blood vessels. These are for the most part capillaries and post-capillary venules, lying in close association with the loose connective tissue which fills the remainder of the canal; occasionally an arteriole is found in a canal. The canalicules of the Haversian systems are extra-vascular, their function presumably being to promote the diffusion of the tissue fluids required for the maintenance of the osteocytes and of the interstitial substance of the bone. In a typical Haversian system (Fig. 101) canalicules branch out from the canal and form a network which includes the lacunae. The canalicules, for the most part, are directed radially, forming the channels of communication between the canal and the successive lamellae of the Haversian system and their lacunae, and circumferentially and longitudinally, providing for intercommunication between the lacunae of the same lamella. The radially arranged canalicules communicate with the broad surfaces of the lacunae; the circumferential and longitudinal canalicules project from the thin edges of the lacunae. In the outermost lamella of an Haversian system the external canalicules loop back into the system; intercommunication between the canalicules of adjacent systems is the exception rather than the rule.

Fig. 105. Cross section of human hip bone in polarized light. The cross sections of three Haversian systems are seen as bright Maltese crosses. 130 ×. Redrawn after Gebhardt.

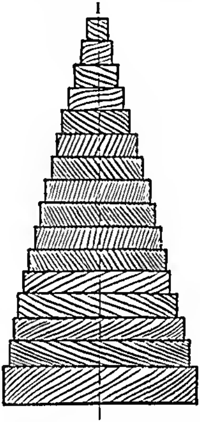

Fig. 106. Diagram of the direction of the fibrils in successive plates of an Haversian system Redrawn and slightly modified from Gebhardt.

In cross sections of an Haversian system, stained for connective tissue fibers, are seen lamellae which are alternately longitudinally and circularly fibrillated. The alternation of perfectly longitudinal and circular lamellae, however, occurs very rarely. More frequently this arrangement is only approximated and the fibrils in all the lamellae run spirally to the axis of the canal. These spirals in adjacent plates cross at various angles and are sometimes perpendicular to one another. The direction of the fibrils within the plates of an Haversian system is shown diagramatically in Fig. 106. The alterna-

tion in the direction of fibrillation in the lamellae causes the optic phenomena seen in cross sections of the systems in polarized light (Fig. 105).

Structure of Compact Bone. Compact bone, e.g., the shaft of a long bone, is made up chiefly of large numbers of Haversian systems. The irregular angular spaces between these systems are filled with the *interstitial* or *ground lamellae* (Fig. 107). Most of these are the remains of the bone or in the marrow cavity are the *canals of Volkmann*, within which are the blood vessels communicating with those of the Haversian canals. They differ in appearance from the Haversian canals in that they are not surrounded by concentrically arranged plates, and that they usually contain larger blood vessels. Haversian canals frequently communicate with the marrow; such intercommunication is also carried out through canals of

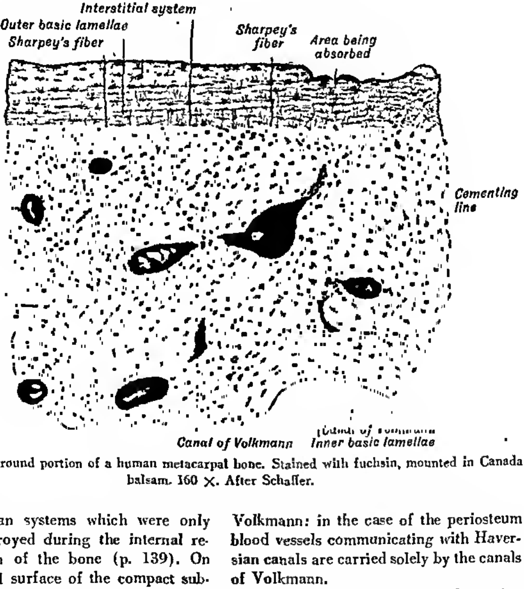

Fig. 107. Ground portion of a human metacarpal bone. Stained with fuchsin, mounted in Canada balsam. 160 ×. After Schaffer.

of Haversian systems which were only partly destroyed during the internal reconstruction of the bone (p. 139). On the external surface of the compact substance, and on the internal surface which forms the wall of the marrow cavity (Fig. 107) are the *basic* or *circumferential lamellae:* these vary in number and are arranged, as the name implies, in the circumference of the bone. Penetrating these lamellae, and opening on the free surface Volkmann: in the case of the periosteum blood vessels communicating with Haversian canals are carried solely by the canals of Volkmann.

Sharpey's Fibers. Compact bone also contains *Sharpey's* or *perforating fibers;* these are collagenous bundles of varying thickness passing from the periosteum through the systems of lamellae in different directions, independently of the ossein fibrils, lacunae and canalicules (Fig. 108,

SF). They are found in those places where, during the formation of a new bone plate, thick collagenous bundles of the surrounding connective tissue become surrounded by bone. When uncalcified they occupy irregular, fairly wide canals in the compact bone substance. When calcified, they appear in sections as prominent, irregular stripes or spots against the background of interstitial substance.

Sharpey's fibers are encountered in the external basic and in the interstitial layers which develop by periosteal ossification. They are not found in the Haversian and internal basic systems. Their number depends on the type of bone and varies greatly; they may appear singly or, as in some of the bones of the skull, in very large numbers. They may be so numerous as to displace much of the interstitial substance and to compress and deform the lacunae (see Weidenreich, 1930).

In addition to Sharpey's fibers it is said that *elastic fibers* also penetrate the bone from the periosteum, and are to be found together with or independently of the collagenous bundles; their occurrence in bone is also denied.

Periosteum, Bone Marrow and Endosteum. Except where it is joined to articular cartilage, bone is covered by the *periosteum*, a special, dense, connective tissue layer. The attachment is very tight on most of the surface of short bones, at the epiphyses of long bones, and where tendons and muscles are attached. This close connection depends mainly on the continuation of dense collagenous bundles from the periosteum into the bone as Sharpey's fibers At such places, too, large blood vessels and nerves enter the bone. Where the periosteum is loosely connected, there are only a few, thin, collagenous bundles and attachment to the bone is largely maintained by small blood vessels.

The periosteum in adults consists of two layers, not sharply defined. The external layer is a network of dense connective tissue containing blood vessels. The deep layer, adjacent to the bone, sometimes called the *cambium layer*, by a fancied analogy with the wood-forming zone of trees, is composed of more loosely arranged collagenous bundles; some of these change direction and enter the bone as Sharpey's fibers. The deep layer also contains spindle-shaped connective tissue cells and a network of thin elastic fibers.

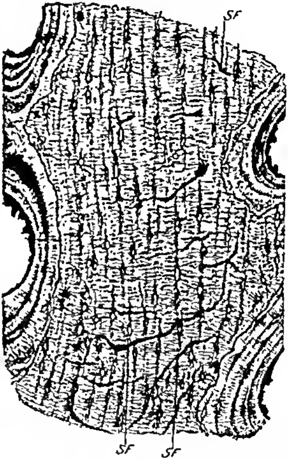

Fig. 108. From a cross section of a human fibula. *SF*, Sharpey's fibers. 160 ×. After Schaffer.

Blood vessels from the external layer enter the deep layer and pass through the canals of Volkmann to the Haversian canals.

In the adult organism the periosteum has no osteogenic function under normal conditions and does not contain osteoblasts. If the bone is fractured, however, the bone-forming potentialities are activated and osteoblasts reappear in the deepest layer of the periosteum (p. 145).

The *endosteum* is a thin connective tis-

sue layer lining the walls of the bone cavities, which are usually filled with bone marrow. It resembles the periosteum in some respects, and is the condensed peripheral layer of the stroma of the bone marrow where it is in contact with bone. All of the cavities of bone, including the marrow spaces within spongy bone, are lined with endosteum after osteoblasts are no longer recognizable; that lining the compact bone of the shaft, however, is more prominent. The endosteum has both osteogenic and hematopoietic potencies.

HISTOGENESIS OF BONE

Bone always develops by a tranformation of embryonic or adult connective tissue into a calcified connective tissue. In embryonic life and in postnatal development, bone arises *de novo* in a relatively small number of areas and growth of bone thereafter is by extension or apposition. Similarly, in the healing of fractures, the bony callus grows by extension from the fractured bone, although not, as a rule, from within the fracture gap. Occasionally, and under certain special circumstances, bone may arise within tissues not connected with the osseous system, and from connective tissue not ordinarily manifesting osteogenic potencies, in which case the process is called *ectopic, heterotopic,* or *metaplastic ossification.*

In embryonic life the greater part of the skeleton is formed by ossification in *cartilage models* of the bones, and growth in length of these bones continues after birth by a similar process of erosion of cartilage and deposition of bone, generally upon a framework of cartilage matrix. This process is called *intracartilaginous or endochondral ossification,* in contrast with simple *intramembranous ossification,* in which bone is formed from connective tissue without intervening stages of cartilage formation and destruction.

Intramembranous Ossification. This process can be studied favorably in the developing bones of the calvarium. The place of origin of the first bone within the embryonic connective tissue is determined, to a large extent, by the course of the blood vessels. Bone first appears between and at equal distance from two neighboring blood vessels and only later spreads toward the vessels. In an area where bone will develop, the connective tissue cells are connected with one another by their processes, and delicate bundles of collagenous fibrils run in all directions between them. The tissue is rather loose and between its cells and fibrils is a semifluid, amorphous substance.

The first signs of bone development in such places are thin bars of dense intercellular substance which run between the cells; they soon become wider and thicker, and often unite with one another to form a network in whose meshes the cells remain. Simultaneously, the cells increase in size and become polyhedral while retaining their numerous processes which are connected with those of the adjacent elements, by which time they become recognizable as osteoblasts (Fig. 109, *O*) or as osteocytes. Singly or in groups the cells become surrounded by the dense interstitial substance, which gradually crowds out the amorphous, semifluid intercellular material (Fig. 109, *B*). Fibrils passing out of the newly formed bone substance continue into those of the surrounding tissue (Fig. 109, *C*). The interstitial substance is explained as a secretion or as a transformation of the protoplasm of its cells; the same explanations are offered for the formation of the interstitial substance in all connective tissue.

When a certain stage in the transformation of the interstitial substance is reached the tissue becomes calcifiable, bone salt is deposited in it, and it is now known as bone. The property of calcifiability is pre-

sumably conferred upon the transformed connective tissue by the osteoblasts. Osteoblasts arise in the early embryo by direct transformation from mesenchymal cells; similarly they may arise in the adult from fibroblasts and reticular cells.

Very soon after the initial stages of formation of bone from connective tissue there are signs of organization; osteoblasts appear on the surface of developing bone in a continuous layer (Fig. 110). Between their lateral surfaces remain spaces through which fibrils pass into the bone from the surrounding connective tissue. To these are added new fibrils formed by the osteoblasts, presumably making up the bulk of the fibrillar structure of the new bone.

Through the activity of the osteoblasts the bone increases in thickness. Successive layers are added, by apposition, to the fibrillar mass which is being calcified while the osteoblasts remain on the exterior surface (Fig. 111). As the bone becomes thicker, some of the osteoblasts which lie on the surface are included, one by one, within its substance; they are the first bone cells or osteocytes, and they lie in the lacunae. They are formed directly from those osteoblasts which have become surrounded by the calcified interstitial substance which they have produced; the possibility of direct transformation of a mesenchymal cell into an osteocyte is not excluded. Most of the osteoblasts continue at the periphery and, with the gradual thickening of the layers of the interstitial substance, they move away from those cells which remain in the lacunae. The osteoblasts maintain a continuous layer on the surface of the plate; as some of them become included in the bone as osteocytes, or as more are required for the formation of bone, new osteoblasts are formed by the transformation of cells from the surrounding connective tissue; mitotic division seldom occurs among osteoblasts themselves.

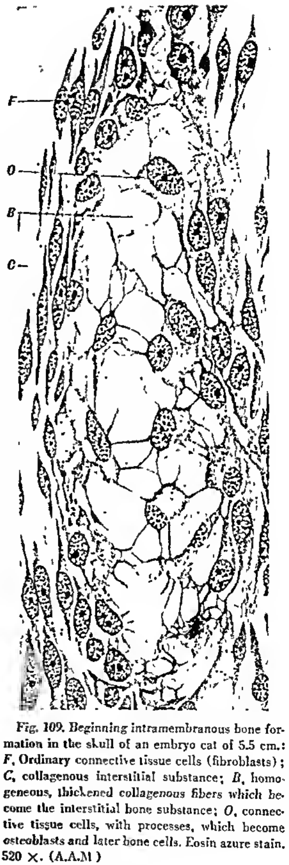

Fig. 109. Beginning intramembranous bone formation in the skull of an embryo cat of 5.5 cm.: *F*, Ordinary connective tissue cells (fibroblasts); *C*, collagenous interstitial substance; *B*, homogeneous, thickened collagenous fibers which become the interstitial bone substance; *O*, connective tissue cells, with processes, which become osteoblasts and later bone cells. Eosin azure stain. 520 ×. (A.A.M.)

Osteoblasts are connected with one another by processes, and osteocytes are osteoblasts included within the ossifying

substance; this explains how the processes of osteocytes penetrate the interstitial bone substance and connect the neighboring cells (compare Figs. 100 and 109). During the transformation of osteoblasts into osteocytes the cytoplasm of the latter even forms a number of new processes. In a mature osteocyte only a few mitochondria remain; these are near the nucleus.

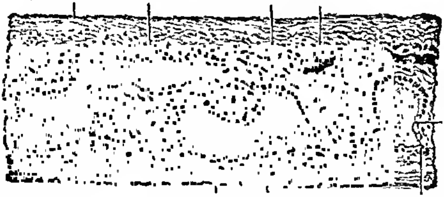

Fig. 110. Cross section through the primordium of the parietal bone of a four-months' embryo. 100 ×. After Schaffer.

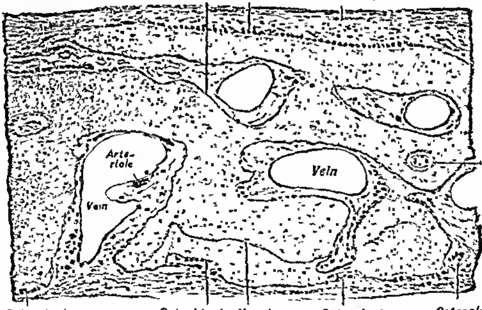

Fig. 111. An analogous section of a six-months' embryo. 100 ×. After Schaffer.

Bone which develops within the connective tissue has a spongy character for a long period. It consists of irregular plates and bars which branch and unite with one another, their fibrils becoming more regularly arranged as the plates thicken. The spaces between the plates are filled with connective tissue rich in blood vessels and dividing cells; this tissue is gradually transformed into myeloid tissue. The connective tissue surrounding a growing mass of spongy bone remains on its surface and gives rise to the periosteum. The osteoblasts which have remained on the surfaces of the bone during its active development assume a fibroblast-like ap-

pearance, and remain as the deepest layers of the periosteum and endosteum; their osteogenic potencies are only recognizable when they are again called upon to form bone, under which circumstances they again assume the morphologic characteristics of osteoblasts.

Intracartilaginous Ossification. In the process of intracartilaginous bone formation, the hyaline cartilage undergoes degenerative changes, is eroded by capillaries accompanied by osteogenic cells, and is replaced by bone which develops in the same manner as in intramembranous ossification. This process is most favorably studied in the zone of endochondral ossification, which is continuous with the epiphyseal cartilage plates of the long bones (Fig. 112), and in which endochondral ossification continues until growth in length of the long bones is complete. In this zone cartilage cells multiply from mother cells and form columns of flattened cells, instead of the irregular isogenous groups found in masses of hyaline cartilage. The cells in a column are separated by thin capsules; adjacent columns are separated by wide, parallel bands of interstitial substance (Fig. 112).

Nearer the zone of ossification, the flat cells in the columns develop small, and then larger vacuoles in their peripheral cytoplasm, causing the cells to swell. The nuclei also swell and lose most of their chromatin, and the cells degenerate. The matrix adjacent to these *vesicular* or *hypertrophic* cartilage cells becomes calcifiable, presumably under the influence of the cells. If there are adequate concentrations of calcium and of phosphate in the blood plasma the matrix then calcifies, especially the broad bands separating, adjacent columns of cartilage cells; this forms the zone of *provisional* or *preliminary calcification*, which bridges and gives rigidity to the gap between hyaline cartilage and spongy bone.

The next stage in intracartilaginous ossification is due to the activity of the connective tissue and blood vessels of the bone marrow. Loops of blood vessels with accompanying connective tissue penetrate the cartilage. The interstitial substance separating the cartilage cells in the columns is dissolved in unknown fashion, and the distended cartilage cells are opened up, so that they are penetrated by the vascular connective tissue. In this way communicating canals arise, whose irregular walls are formed by the deeply staining and calcified cartilage matrix; the canals are filled with blood vessels and loose connective tissue; they lengthen as new capsules are opened up. Most of the vesicular cartilage cells perish during penetration of their capsules by the capillaries; a few of them may survive and become osteoblasts.

The process just described is dependent, for its orderly progress, upon the formation of the zone of provisional calcification, which advances just ahead of the penetration of the cartilage by capillaries and removal of the cartilage cells. If calcification in the cartilage matrix fails, owing to a deficiency of bone minerals, cartilage removal ceases; if multiplication of cartilage cells in columns continues, as is usually the case, the epiphyseal cartilage plate increases greatly in thickness. This, together with the appearance of uncalcified osteoid tissue in excessive amounts (see next paragraph), constitutes the histological picture characteristic of *rickets* (Fig. 120).

When the vascular connective tissue penetrates the capsules, those connective tissue cells touching the cartilage matrix turn into a layer of osteoblasts. Then, between the osteoblasts and the cartilage matrix, the latter of which remains as a scaffolding upon which new bone is deposited, a thin, new layer of tissue appears, gradually thickens, and surrounds the contours of the cartilage bars (Fig. 101). This tissue is calcifiable when it is laid

substance; this explains how the processes of osteocytes penetrate the interstitial bone substance and connect the neighboring cells (compare Figs. 100 and 109). plates and bars which branch and unite with one another, their fibrils becoming more regularly arranged as the plates thicken. The spaces between the plates are

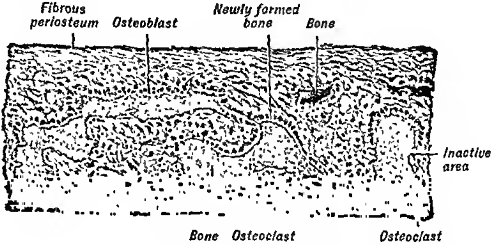

Fig. 110. Cross section through the primordium of the parietal bone of a four-months' embryo. 100 ×. After Schaffer.

During the transformation of osteoblasts into osteocytes the cytoplasm of the latter even forms a number of new processes. In a mature osteocyte only a few mito- *filled* with connective tissue rich in blood vessels and dividing cells; this tissue is gradually transformed into myeloid tissue. The connective tissue surrounding a

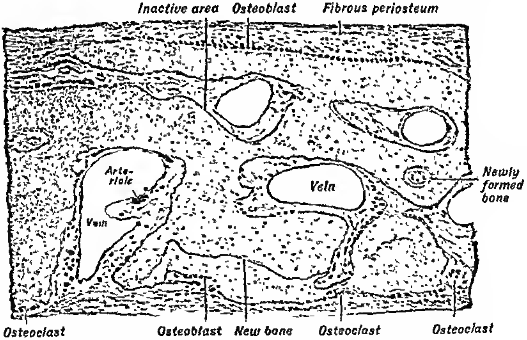

Fig. 111. An analogous section of a six-months' embryo. 100 ×. After Schaffer.

chondria remain; these are near the nucleus.

Bone which develops within the connective tissue has a spongy character for a long period. It consists of irregular growing mass of spongy bone remains on its surface and gives rise to the periosteum. The osteoblasts which have remained on the surfaces of the bone during its active development assume a fibroblast-like ap-

ure becomes general, and osteoid tissue appears in excess, the condition is known as *rickets* or *osteomalacia* (p. 149). The calcification of new bone is best demonstrated in undecalcified sections, in which the bone salt is positively stained.

From this point, bone is formed by the osteoblasts just as in intramembranous bone formation. In quite the same fashion, the layers of the interstitial substance become thicker and surround isolated osteoblasts, transforming them into osteocytes. The spongy endochondral bone at this time consists of various sized trabeculae, covered by osteoblasts, with a few osteoclasts, and containing the remains of cartilage matrix in their interior; the latter serve to differentiate bone formed by endochondral ossification from that arising by the intramembranous process alone. The wide spaces between the plates are filled with hematopoietic bone marrow.

Internal Reconstruction of Bone. Bones developing in cartilage increase in length by endochondral ossification and radially by the deposition of new periosteal bone. The process of increase in size is complicated by the necessity of weight bearing throughout the whole period of growth, requiring continuous internal reconstruction of the growing bones. The final result is an increase in strength, as well as size, of the bones, together with the provision of the large cavities lodging the bone marrow.

With the first appearance of bone, wherever formed, a destructive process also appears, generally associated with the presence of osteoclasts (p. 126). The relation of the osteoclasts to the resorption or dissolution of bone is uncertain. It is commonly believed that they produce a substance which dissolves the bone, but there is no direct evidence to support this belief. Osteoclasts are usually, but not always, seen where bone is dissolving, and are frequently found in deep grooves, *Howship's lacunae*, which have the appearance of having been eroded in the bone. Or an osteoclast may surround the free edge of the end of a trabecula of bone undergoing dissolution. The possibility that the osteoclast has a phagocytic function seems to be slight; both organic matrix and inorganic bone salt are resorbed simultaneously, but neither cellular débris nor bone salt can be demonstrated in the osteoclasts, although under certain circumstances both appear in the macrophages of the bone marrow.

When resorption of bone ceases in a particular location the osteoclasts disappear, most of them becoming either osteoblasts or reticular cells, depending upon whether new formation of bone is to follow. Osteoclasts are but rarely seen to degenerate. This reconstruction of bone is coordinated with the existing local mechanical conditions. The direct causes determining whether adjacent cells in a growing bone become either osteoblasts or osteoclasts are unknown.

A greatly diminished rate of reconstruction of bone, the "physiological turnover," continues throughout adult life, and is associated with the maintenance of the level of calcium in the plasma. In fact, calcium can be made available to the blood from bones only by destruction of osseous tissue; simple decalcification of bone by solution of its minerals in the body fluids (*halisteresis*) in all probability does not occur. When, in adult life, the skeleton is called upon to furnish large amounts of calcium, as in pregnancy, the turnover of bone may be greatly accelerated. If, under these circumstances, there is not sufficient calcium in the diet to calcify the new osseous tissue formed, the condition known as *osteomalacia* (*adult rickets*) follows.

Formation of Haversian Systems. In parts of the skeleton spongy bone becomes transformed into compact bone. In the irregular, communicating cavities of the spongy bone, filled with bone marrow, the amount of marrow between the spic-

down and, under favorable conditions, begins to calcify as it is deposited, and not a necessary step in the formation of bone, but there may be a lag in calcifica-

Fig. 112. The zone of endochondral ossification in a longitudinal section through the tibia of a cat embryo of 12 cm. Eosin-azure stain. 220 ×. (A.A.M.)

thus acquires the characteristics of osseous tissue, or bone. An intermediate stage of uncalcified osseous tissue, or *osteoid*, is tion, even under physiological conditions, owing to a local failure in the supply or transport of bone mineral. When this fail-

The external shape of the cartilage model suggests in general the future bone; in it a diaphysis, with an epiphysis at each end, may be distinguished very early. Externally the cartilage model is covered by a perichondrium of closely packed, embryonic connective tissue cells. Bone formation begins within a ring-shaped area surrounding the center of the diaphysis. The perichondrium is called the periosteum as soon as it begins to form bone and the process is accordingly called "periosteal ossification," and is of the intramembranous type (p. 134). The cells of the perichondrium adjoining the cartilage increase in size and become osteoblasts. Between them appear very thin and later thicker bone lamellae with bone cells (Fig. 115). These lamellae form a bone ring, the *periosteal bone band* or *collar*, which surrounds the middle of the diaphysis of the cartilage. This band is a spongy bone network, through whose meshes the connective tissue of the periosteum continues in direct contact with the cartilage.

Before or shortly after the bone collar appears, the cartilage tissue inside of the diaphysis changes markedly; the cells swell into vesicles and the interstitial substance between them becomes thinner (Fig. 115) and calcified. Through the spaces in the periosteal band the connective tissue of the periosteum, together with the blood vessels, penetrates the transformed cartilage at one or several places (Figs. 116 and 117). The capsules of the vesicular cartilage cells are quickly opened for a long distance (p. 137), and become filled with embryonic bone marrow with its thin-walled blood vessels In

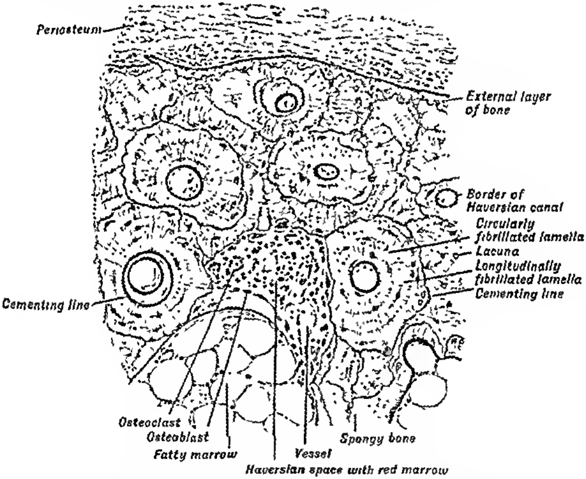

Fig. 114. Cross section of the second phalanx of a human middle finger showing replacement of spongy bone by compact bone 110 ×. After Schaffer

ules of bone decreases as the osteoblasts covering the bone produce layer after layer of concentric bone plates or lamellae. This process continues until all that remains of the former marrow cavity is a comparatively narrow canal containing the blood vessels surrounded by a very little of the bone marrow. In this manner systems of concentric bone lamellae, called *primitive Haversion systems*, are formed.

Then, at some places, the bone substance begins to dissolve, a process which may include parts or all of the newly formed primitive Haversian systems as well as the periosteal bone, and which is associated with the presence of osteoclasts. Haversian canals, and in an increase in the compactness of the bone.

This process of destruction and construction continues actively until the bones approach adult size, after which reconstruction continues throughout life, but at a sharply reduced intensity. It is upon this reconstruction that the complicated structure of the mature compact substance with its Haversian and intermediate systems depends. As a bone in*creases in thickness the periosteum* and the endosteum lay down successive layers of *basic* or *circumferential lamelloe* on the surfaces of the bone. During reconstruction of the bone these lamellae also

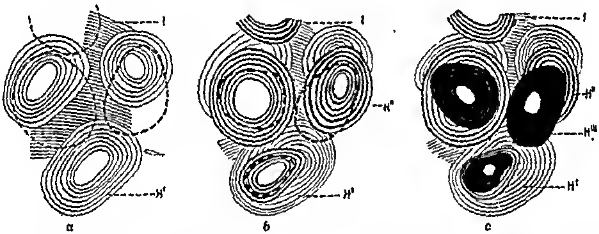

Fig. 113. Diagram showing stages in the formation of three generations of Haversian systems, *H'*, *H''*, *H'''*; *I*, interstitial lamellae. Slightly modified after Prenant.

Wide cylindrical cavities filled with blood vessels and with embryonic bone marrow are formed anew. Then the destruction of bone ceases, the activity of the osteoblasts begins, and concentric systems are once more laid down on the walls of the cavities. These are the Haversian systems of *the second* generation. The dissolution may be renewed in adjacent areas of the bone, to be followed by the formation of Haversian systems of the third generation (Figs. 113, 114). Haversian systems are always formed by apposition of bone on the inner surfaces of lamellae of bone surrounding blood vessels; this results in a progressive decrease in the size of the undergo destruction and replacement by Haversian systems. The intermediate systems, then, include the remains of these lamellae, of which those of periosteal origin contain Sharpey's fibers, and portions of former, partially destroyed Haversian systems of various generations.

Development of Bones as a Whole. Most of the bones of the skeleton are first laid down in the embryo in hyaline cartilage; this group includes all the bones of the thorax, the limbs, the greater part of the bones of the skull, and the hyoid bone. The ossification of these cartilage models proceeds typically in the long bones of the limbs.

this manner a cavity with irregular walls is formed in the cartilage of the interior of the diaphysis in the area surrounded by the periosteal band (Fig. 115, middle; Fig. 118). This constitutes the *primary bone marrow cavity*, filled with embryonic bone marrow. The periosteal collar performs an important function by bridging some of the cartilage cells also become osteoblasts. In other places osteoclasts are formed where the newly formed bone is being resorbed (Fig. 117).

With the continued growth of cartilage in the epiphyses, the entire cartilage model increases in size. The mass of the periosteal as well as of the endochondral

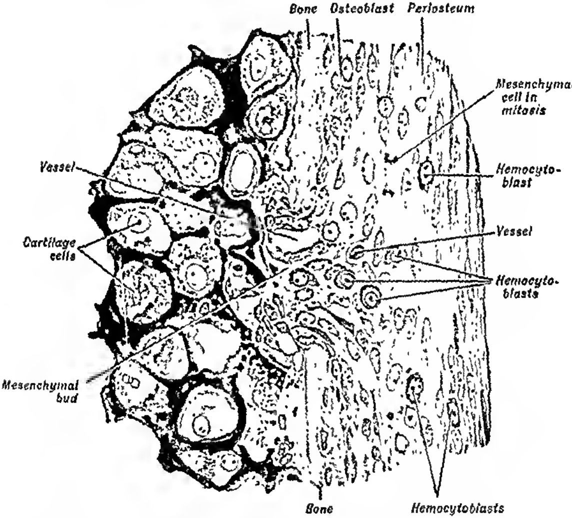

Fig. 116. Part of a longitudinal section through the middle of the diaphysis of the femur of a 25 mm. human embryo. Mesenchyme with vessels entering calcified cartilage through an opening in the periosteal bone collar. *Eosin-azure stain.* 560 ×. (A.A.M.)

the gap which would otherwise result from the formation of this cavity; it is the primitive shaft of the bone. Scattered between the elements of the marrow are angular remains of calcified cartilage Some of the cells of the embryonic marrow become osteoblasts, which come in contact with these cartilaginous remains and surround them with layers of bone; bone in the diaphysis also grows progressively. The periosteal bone band widens toward the epiphyses, and is demarcated from the spongy endochondral bone by a thin refractile line. The endochondral bone may also be distinguished by the remains of deeply staining cartilage matrix within its trabeculae. At a much later period, in man usually during the first

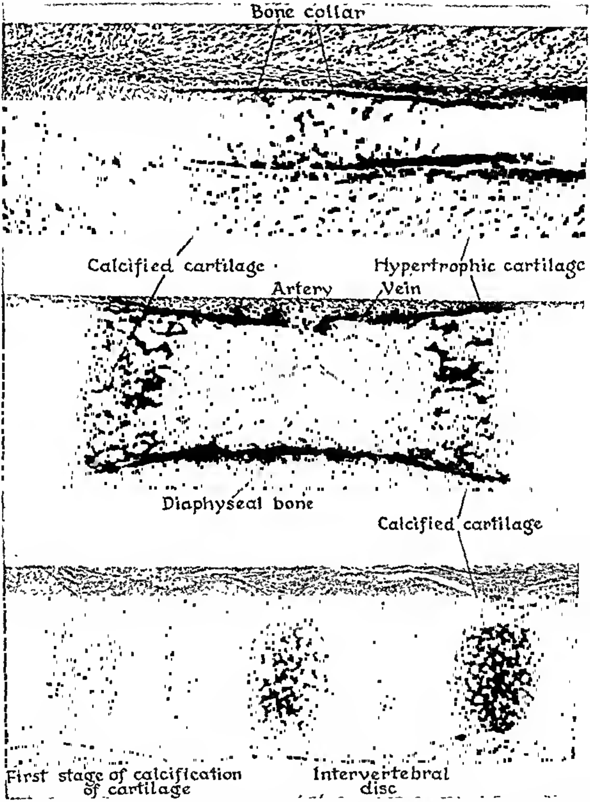

Fig. 115. Photomicrographs showing several stages of bone formation in developing rats. All three figures are from formalin fixed, undecalcified sections stained with silver nitrate to show bone salt (black). Upper figure is a longitudinal section through second rib of eighteen-day rat embryo; [illegible]

vertebrae in twenty-day rat embryo. 117 ×, 63 ×, and 57 × respectively. (Bloom and Bloom.)

replaces the cartilage, the cartilage matrix separating the columns of cartilage cells becomes covered with osseous tissue, forming the mass of spongy bone called the metaphysis. This bone normally undergoes extensive reorganization and thinning out as the growth process passes it by; two parts of it become recognizable, the *primary* and *secondory spongiosa*, being differentiated by the fact that the secondary spongiosa has undergone reconstruction. As a part of this reconstruction the tips of trabeculae of the spongiosa are continually undergoing resorption, with the result that the spongiosa tends to remain constant in length. The total picture in the growth zone, then, is that of an epiphyseal cartilage disc, a zone of provisional calcification, within which cartilage cells are being opened and destroyed, and a zone of spongy bone; all of these zones remain at approximately constant dimensions, while the shaft of the bone increases in length, owing to the fact that the proliferating structures, the growth apparatus, constantly grow away from the diaphysis.

At first the only bone formed is of the spongy type, of periosteal and endochondral origin. Then, the internal reconstruction begins in the periosteal bone and leads to the development of compact bone. In the diaphysis it finally forms a very thick layer, which tapers gradually toward the epiphyses. Inside the central portion of the diaphysis, and extending toward the epiphyses, an extensive dissolution of bone begins, which is not compensated by a corresponding formation of new bone; in this manner the *definitive bone marrow cavity* arises. In a completed bone it occupies the entire diaphysis and continues into the spaces of the spongy substance toward the epiphyses.

In short bones developing in cartilage, a central point of endochondral ossification appears and progresses from the center to the periphery. This continues until only a thin cartilage layer remains. When the cartilage no longer regenerates and is completely used up, the surrounding layer of connective tissue becomes the periosteum and begins to deposit upon the exterior of the endochondral spongy bone a layer of periosteal bone of varying thickness. This later becomes compact bone.

In flat bones which develop in cartilage (scapula), periosteal ossification is followed by endochondral bone formation just as in the long bones. The bones of the calvarium, the sides of the skull and almost all of the facial bones develop directly from connective tissue. In a limited region of this tissue a small center of spongy bone is formed. Its lamellae develop radially from this point of ossification in one place; such a growing bone consists of a solid mass in its interior and thin, bony rays at its periphery. The layers of connective tissue which cover the surfaces differentiate into periosteum and increase the thickness of the bone by apposition. The peripheral layers of the bone become compact substance through internal reconstruction. In the central layers, dissolution outstrips the formation of new bone so that in the course of time there is formed a spongy substance with very wide bone marrow spaces—diploë.

The lower jaw is an example of a special mode of ossification, for its model, although formed in the embryo of cartilage (*Meckel's cartilage*), does not undergo ossification, but simply serves as a surface for the deposition of bone by connective tissue. The cartilage is later resorbed.

Repair of Bone. Following a fracture there are first the usual reactions of any tissue to severe injury, including hemorrhage and organization of the clot by ordinary granulation tissue, the *pro-callus*. The granulation tissue becomes dense connective tissue, and cartilage and fibrocartilage develop within it, forming the *fibrocartilaginous callus*, which fills the

few years of postnatal life, *ossification centers* appear in the epiphyses, until then formed of hyaline cartilage, and the process of endochondral ossification is repeated in these areas.

The epiphyseal cartilage plates (see p. 124) between the epiphyses and the diaphysis are temporary formations which serve for the growth in length of the bone. Multiplication of cartilage cells, arranged in columns, occurs from the epiphyseal aspect of the disc, removal of mature or hypertrophic cells from the diaphyseal aspect. Under normal conditions of growth these two processes balance one another, and the disc remains at approximately constant thickness. Growth in length results from the fact that the cartilage cells continually grow away from the shaft, being replaced by bone as they recede; the net effect is an increase in length of the shaft. When proliferation of the cartilage ceases, at the end of the period of growth, the cartilage plates or discs are entirely replaced by bone (*closure of epiphyses*), the epiphyses unite with the diaphysis, and longitudinal growth of the bone is no longer possible (Fig. 118).

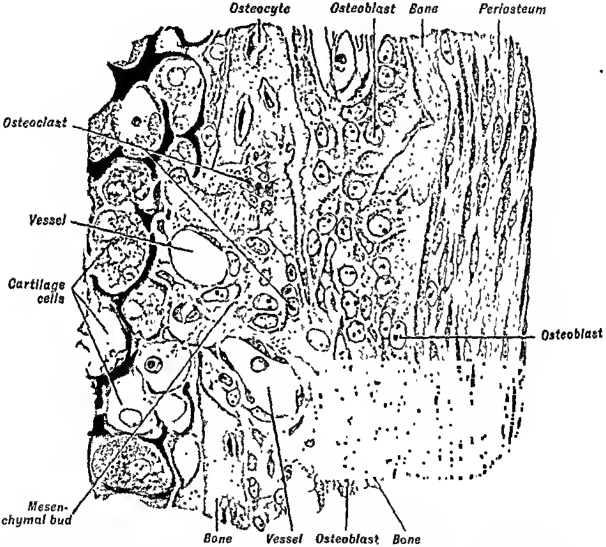

Fig. 117. Similar place as in Fig. 116 from the humerus of a human embryo of eight weeks. The process of ossification has advanced slightly farther than in Fig. 116. Eosin-azure stain. 560 ×. (A.A.M.)

The contribution of each of the two epiphyses of a long bone to its growth may differ markedly; growth in length of the femur takes place mainly at the distal epiphysis.

As the growing bone advances into and

gap between the ends of the fragments. The new bone, which will ultimately unite the fragments, begins to form at some distance from the fracture line, originating from the deeper layers of the periosteum and endosteum, and invading the fibrocartilaginous callus at its periphery (Bast, Sullivan and Geist). It extends centripetally, replacing the tissues of the callus with new bone, the *bony callus*, which is calcifiable as it is laid down and, under favorable conditions, calcifies as it is formed. Ossification of the callus, then, like intracartilaginous bone formation, is essentially a process of replacement of the earlier tissue by bone, only enough of the first tissue remaining to furnish a framework for the deposition of the new bone. *Bony union* of the fracture is accomplished when the new spongy bone, invading the callus from the periosteum of the two fragments of bone, makes contact and unites. Following this there is reorganization, with resorption of excess bone, and internal reconstruction, resulting finally in bridging the gap with compact bone. The fate of the cells of the fibrocartilaginous callus needs further study.

Ectopic Ossification. In the processes above described bone has been formed from connective tissue, with the transformation of fibroblasts and reticular cells into osteoblasts, osteocytes and osteoclasts; the return of these cells to fibroblasts and reticular cells has also been described. All of the processes described have in common the fact that bone has developed only in connection with the osseous system—the skeleton. The influences under which ordinary connective tissue gives rise to bone in the embryo are but little understood, but it is clear that previously undifferentiated connective tissue cells are capable of transformation to the cells characteristic of bone.

It would also appear that once cells have exhibited osteogenic potencies these potencies are readily evoked for an indeterminate period after the cells have returned to an indifferent morphologic state. Thus in the healing of fractures, as described above, cells in the deepest layers of the periosteum and endosteum, under the stimulus of trauma, reassume the form of osteoblasts and once again are actively engaged in osteogenesis. Moreover, cells grown from bone in tissue culture, and having lost the morphologic characteristics of osteoblasts, once again form bone when implanted into the anterior chamber of the eye (Fig. 119).

Furthermore, under certain conditions bone may be formed spontaneously from connective tissue not in association with the skeleton. This ectopic ossification has been described in such diverse locations as the pelvis of the kidney, in the walls of arteries, in the eyes, in muscles, and in tendons From this it may be inferred that many types of connective tissue have latent osteogenic potencies, which are exhibited only rarely away from the skeletal system. This conclusion is supported by experimental production of bone in connective tissue following ligation of the renal artery and vein (Sacerdotti and Frattin), following transplantation of bladder epithelium (Huggins), and following the injection of alcoholic extracts

periosteal bone collar before the development of calcified cartilage, *c*, or after it, *d*; *e*, vascular mesenchyme has entered the calcified cartilage matrix and divided it into two zones of ossification, *f*; *g*, blood vessels and mesenchyme enter upper epiphyseal cartilage; *h*, epiphyseal ossification center develops and grows larger; *i*, ossification center develops in lower epiphyseal cartilage; *j*, the lower and *k*, the upper epiphyseal cartilages disappear as the bone ceases to grow in length and the bone marrow cavity is continuous throughout the length of the bone. After the disappearance of the cartilage plates at the zones of ossification, the blood vessels of the diaphysis, metaphysis and epiphysis intercommunicate.

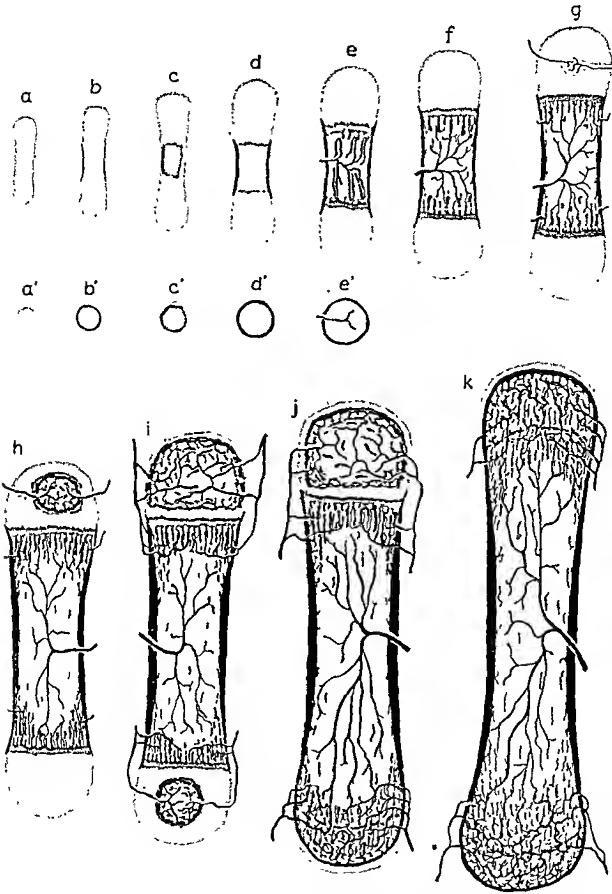

Fig. 118. Diagram of the development of a typical long bone as shown in longitudinal sections. *Pale blue*, cartilage; *purple*, calcified cartilage; *blue*, bone; *red*, arteries. *a'*, *b'*, *c'*, *d'*, *e'*, are cross sections through the centers of *a*, *b*, *c*, *d*, *e* respectively. *a*, cartilage model; *b*, appearance of the

gap between the ends of the fragments. The new bone, which will ultimately unite the fragments, begins to form at some distance from the fracture line, originating from the deeper layers of the periosteum and endosteum, and invading the fibrocartilaginous callus at its periphery (Bast, Sullivan and Geist). It extends centripetally, replacing the tissues of the callus with new bone, the *bony callus*, which is calcifiable as it is laid down and, under favorable conditions, calcifies as it is formed. Ossification of the callus, then, like intracartilaginous bone formation, is essentially a process of replacement of the earlier tissue by bone, only enough of the first tissue remaining to furnish a framework for the deposition of the new bone. *Bony union* of the fracture is accomplished when the new spongy bone, invading the callus from the periosteum of the two fragments of bone, makes contact and unites. Following this there is reorganization, with resorption of excess bone, and internal reconstruction, resulting finally in bridging the gap with compact bone. The fate of the cells of the fibrocartilaginous callus needs further study.

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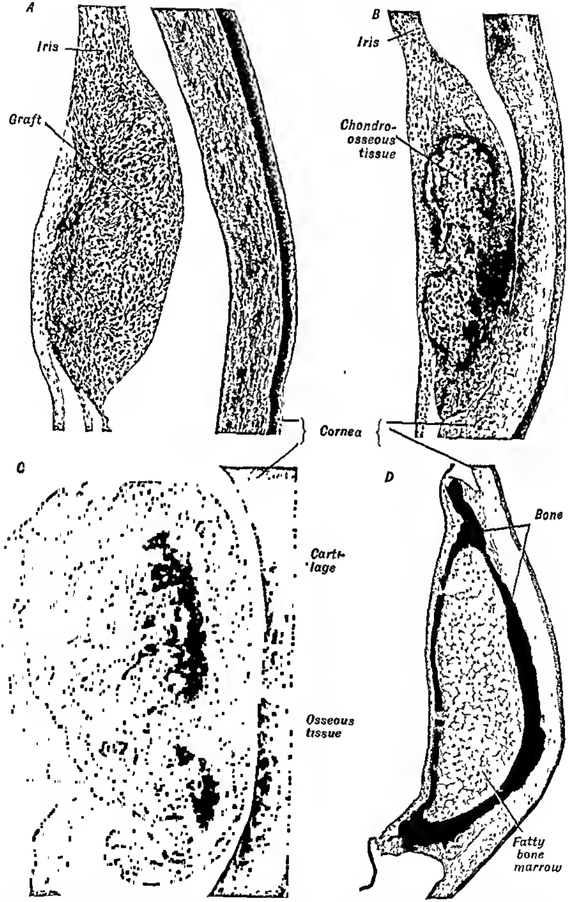

Fig. 119. Four stages in the development of tissue cultures of bone grafted into the anterior chamber of the eye. Fragments of tibiae of twelve-day rats were cultured in rat plasma and embryo extract. The outgrowths were removed from the original explant at the end of four days *in vitro* and were transplanted to the anterior chambers of the eyes of normal rats. At the time of implantation

of bone into muscle (Levander; Annersten). In the latter case there is evidence (Heinen) that alcohol alone, if sufficiently dilute to avoid production of necrosis, frequently induces osteogenesis in muscle, and that it shares this ability with other irritating substances.

Many attempts have been made to utilize the osteogenic potencies of periosteum by transplantation of this tissue to areas in which it is desired that new bone be formed. In general, periosteum transplanted away from bone does not form bone, although it has been stated that if small fragments of bone adherent to it are transplanted with it, osteogenesis may follow, even though the fragments of bone themselves undergo necrosis. Reticular cells within the orbit of advancing bone, e.g., in the formation of medullary bones in birds (p. 151) assume the form of osteoblasts before they actually join in the process of osteogenesis. These observations suggest that the presence of bone itself may be an important aid in the eliciting of osteogenic potencies.

Histophysiologic Remarks. The mechanism of the deposition of calcium in bone is still incompletely understood. At least two factors, local and humoral, are involved. The humoral factor is related to the supply of minerals in the fluids of the body, and to the solubilities of the difficultly soluble salts of calcium and phosphate; it may be defined in terms of the concentrations of these substances in the blood. But the local factor, which determines the occurrence and specific localization of the deposition of bone salt, when adequate concentrations of calcium and of phosphate are present in the blood, is not understood.

When calcification fails in growing animals or young children the osseous tissue continues to grow, but the new uncalcified, but calcifiable tissue is known as *osteoid.* This failure of calcification to keep pace with growth is known as *rickets,* and is usually associated with a diminished concentration of phosphate in the blood plasma. The preventive and curative action of *sunlight* and of *vitamin D* in rickets is generally attributed to increased absorption of calcium and phosphate from the gastro-intestinal tract. That rickets is not due to failure in the local mechanism is shown by the fact that the cartilage matrix of rachitic bone calcifies readily, either *in vivo,* or *in vitro,* when the surrounding medium is supplied with the necessary minerals in adequate concentrations (Fig. 120). *Phosphatase* is present in osteoblasts, but not in osteoid tissue; the staining reactions of these cells also indicate that they are rich in ribosenucleic acid: it is suggested that these substances are associated with the formation of the bone matrix by osteoblasts, rather than with calcification.

The interstitial substance of bone acts as a store for calcium, there being a constant interchange of this substance between the blood and the bones, with the result that the calcium ion concentration in the plasma remains approximately constant. Calcium, when once laid down in the form of bone salt, can be made available to the blood only by destruction of osseous tissue, including its organic matrix, chiefly from the spongy bone near

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the outgrowths contained no bone or cartilage *A*, Section of culture after one and one-half days in the eye No cartilage or bone. *B*, Section of a culture after two and one-half days in the eye; chondro-osseous tissue has developed from the culture. *C*, Section of a culture after six days in the eye. Much bone and cartilage have developed from the culture. Primitive marrow is beginning to appear. *D*, Section of a culture after four months in the eye A "bone" with central fatty marrow has developed from the irregularly arranged bone and cartilage of the early stages. *A*, *B*, *C*, Zenker-formol, hematoxylin-eosin-azure II. Photomicrographs 80 ×. *D*, Formalin fixation, silver nitrate-hematoxylin-eosin. Photomicrograph 32 ×. Courtesy of J. H Heinen

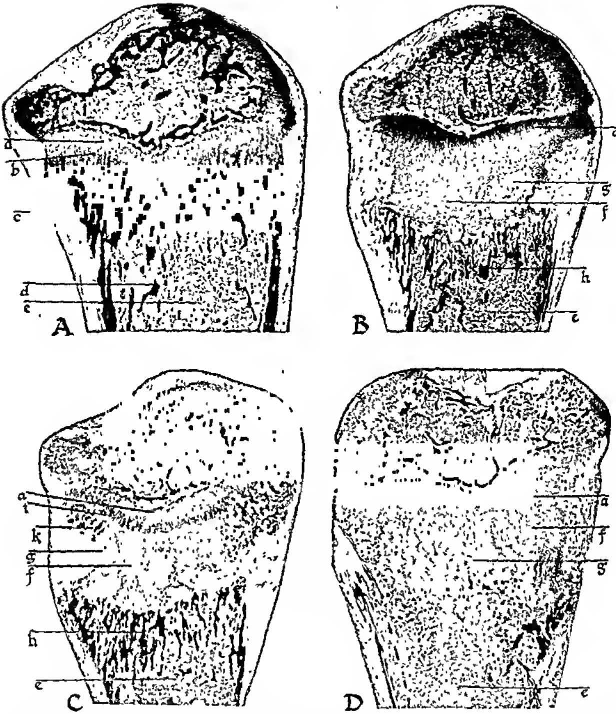

Fig. 120 The head of the tibia in experimental rickets in rats. All sections from undecalcified bones stained with $AgNO_3$ (von Kóssa) to show calcification and counterstained with H and E. All rats weaned to experimental diet at twenty-one days. A, Normal control, age thirty-one days, Bills' stock diet. B, Littermate of A, age thirty-one days, same diet with addition of 2% $BeCO_3$. C, Age fifty-seven days, Bills' stock diet with addition of 1% $BeCO_3$, given daily intraperitoneal injections of mixture of phosphates of sodium for last seven days Note calcified cartilage matrix and beginning healing. D, Age sixty-two days, rickets due to high calcium low phosphate diet (Steenbock-Black). *a*, Proliferating cartilage; *b*, zone of provisional calcification, *c*, trabeculae of primary spongiosa; *d*, secondary spongiosa; *e*, bone marrow; *f*, hypertrophic cartilage; *g*, osteoid tissue; *h*, trabeculae calcified before onset of rickets; *i*, calcification preparatory to healing; *k*, healing with calcification of new bone Photomicrographs 15 ×. Courtesy of F. C. McLean.

the epiphyses of the long bones. The rate of the resorptive process is regulated by the *parathyroid hormone*, which has no effect upon the deposition or calcification of bone.

In birds, an entire new system of *med-*

ullary bone, produced chiefly by an outgrowth from the endosteal lining of the shafts of the long bones, is formed during the egg-laying cycle, or may be induced by the administration of *estrogens,* and osteoclasts are prominent during this resorptive stage. Mice react to the administration of estrogens in much the same way as do birds; endosteal bone formation has not been reported in rats, in

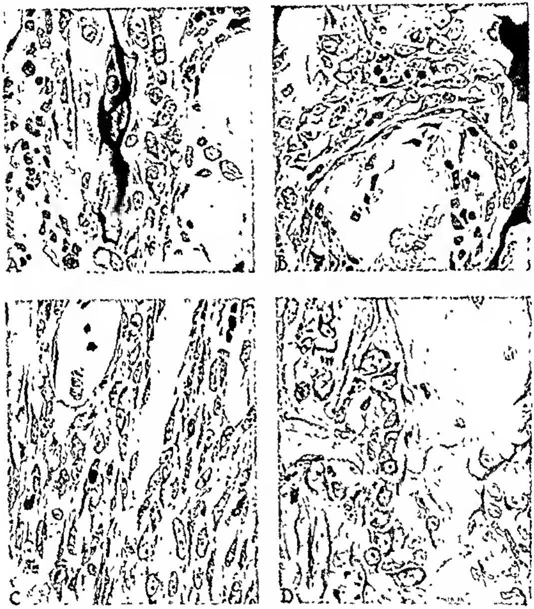

Fig 121. The effect of large doses of parathyroid extract on the proximal epiphysis of tibia of rats. The sections are from four members of a seven-weeks litter. A, Normal control, with prominent osteoblasts; B, nine hours; C, twenty-four hours; D, ninety-six hours after the injection of 1000 units of parathyroid extract B, Extensive development of osteoblasts into fibroblast-like cells. C, Bone marrow and osteoblasts have been replaced by densely packed fibroblasts; D, the fibrous tissue has been replaced by newly formed bone, many of its fibroblasts having changed into osteoblasts. 505 ×. Hematoxylin eosin-azure II. After preparations of McLean and Bloom.

serves to accumulate calcium to be used in the formation of the egg shell. When the egg shell is being calcified, calcium is made available by destruction of the medullary bone, including its organic matrix; which estrogens have the effect of inhibiting the normal resorption of the spongiosa during growth by endosteal ossification. This latter effect results in a greatly elongated and dense spongiosa, contain-

ing cores of deeply stained cartilage matrix, thus resembling the findings in *marble bone disease.*

The growth of bone is markedly influenced by the *growth hormone* of the anterior pituitary; hypophysectomy results in cessation of endochondral ossification, administration of growth hormone reinitiates growth, and if continued for a sufficiently long time, may result in *gigantism.* The *thyroid glands* influence the growth of bone, but not specifically.

In *hyperparathyroidism* bone is extensively resorbed, and is replaced by fibrous tissue containing large numbers of osteoclasts; this resembles the pathological picture of *osteitis fibrosa* (*von Recklinghausen's disease*). When large doses of parathyroid hormone are given to animals, changes in the bones are profound. Within a few hours many osteoblasts die, although the majority change into fibroblasts or osteoclasts or become phagocytic. There is widespread necrosis of the elements of the bone marrow and, at least in certain species, of the osteocytes. The calcium-containing trabeculae are rapidly resorbed and replaced by fibrous tissue. Salts of calcium have not been demonstrated within the osteoclasts, but have been found extracellularly as well as in the macrophages of the marrow. Recovery occurs when large numbers of osteoblasts develop from fibroblasts, and new bone, formed intramembranously, replaces the fibrous tissue (Fig. 121).

In long-standing deficiency of calcium and of vitamin D, especially when aggravated by pregnancy, the bones of adults contain much uncalcified osteoid tissue, and their mineral content is greatly diminished—a condition known as *osteomalacia* (*adult rickets*). The diminution in calcium content is due to failure of calcification of new bone formed in the turnover of this tissue, rather than to simple decalcification of previously calcified bone. In osteomalacia, and in the form of rickets induced experimentally by deprivation of calcium, the parathyroid glands are enlarged; they are much less affected in the rickets associated with deficiency of phosphate.

In *osteoporosis,* a term generally understood to mean an increase in the relative size of the Haversian canals with a corresponding decrease in the mass of the compact bone substance, the total mineral content of the bones is diminished; it has not been shown that the mineral content of the bone substance itself is lessened in this condition, which occurs in elderly people, and more especially in women after the menopause. In *Paget's disease,* little understood, the bones are both thickened and softened, resorption and apposition of bone occurring simultaneously. In this condition the blood calcium and the parathyroid glands are normal.

Deficiency of *vitamin C* leads to profound changes in tissues of mesenchymal origin, producing the condition known as *scurvy* (*scorbutus*), which has been characterized as an inability of the supporting tissue to produce and maintain intercellular substance. In bone this results in destruction of the collagen of the matrix, with extensive reparative proliferation of fibroblasts. Deficiency of *vitamin A* results in a diminution in the rate of growth of the skeleton, without a corresponding retardation of growth of the central nervous system; the resulting damage to the central nervous system is regarded as mechanical, owing chiefly to the discrepancy in the relative sizes of the spinal cord and the vertebrae. Excessive administration of vitamin A accelerates remodelling of bone (Wolbach).

JOINTS AND SYNOVIAL MEMBRANES

Bones are joined to one another by connective tissue structures which permit varying degrees of movement between the adjoining bones. Such structures are

called joints or articulations. These present extreme variations in character which depend primarily upon the type of bones which are joined and the varying degrees of motion permitted by the articulation. Thus, in some cases, as in the skull, the joints are immovable, and the connected bones are separated only by a very thin connective tissue layer, the sutural ligament. Other joints are slightly movable, as the intervertebral articulations Here the succeeding vertebrae are joined to one another by dense fibrous tissue and cartilage. Still other bones are freely movable upon one another and here the bones are completely separated by cartilage and fibrous capsules.

Joints in which there is little or no movement are called *synarthroses*. There are three types of these: If the connection between the bones is of bone, it is a *synostosis;* if of cartilage, a *synchondrosis*, and if of connective tissue, a *syndesmosis* Joints which permit free movement of the bones are called *diarthroses*.

In the diarthrodial joints there is a cavity. Because this was thought by some to have a continuous lining of flattened, epithelium-like cells, the tissue was called "mesenchymal epithelium" However, the walls of the joint cavities are composed of a dense connective tissue whose cells are irregularly distributed and seldom suggest epithelium in arrangement. Occasionally, small amounts of cartilage and all transitions between the cartilage cells and the joint or synovial cells can be found.

The articular surface of the bones is covered with hyaline cartilage. Where the opposing cartilages touch, they are not covered with dense connective tissue, but at their bases a small area of perichondrium is reflected backward into the membrane of the joint capsule. At this point there are many cartilage cells extending into the synovial membrane. As is true of most of the cartilage of the body, the articular cartilages contain no blood vessels; it is generally believed they are nourished by osmosis from the surrounding tissues. The articular cartilages are intimately adherent to a layer of compact bone which lacks Haversian systems and has quite large lacunae, said to be free of canalicules

Fig. 122. Hip joint of man; the synovial membrane has relatively few cells; between them are collagenous fibers. *fz*, Cell lying free on the surface; *a*, swollen cellular process on the surface; *fa*, processes projecting freely above the surface. After Hammar.

Most of the joint capsules are composed of two fairly distinct layers. The external consists of dense fibrous tissue and is called the *fibrous layer*. The inner is the *synovial layer* which is more cellular and is thought to secrete the viscid, colorless liquid of the joint cavity. However, the joint membrane exhibits many variations in structure. The synovial layer is sometimes thrown into marked folds which may project for surprising distances into the cavity. The larger of these folds fre-

ing cores of deeply stained cartilage matrix, thus resembling the findings in *marble bone disease.*

The growth of bone is markedly influenced by the *growth hormone* of the anterior pituitary; hypophysectomy results in cessation of endochondral ossification, administration of growth hormone reinitiates growth, and if continued for a sufficiently long time, may result in *gigantism.* The *thyroid glands* influence the growth of bone, but not specifically.

In *hyperparathyroidism* bone is extensively resorbed, and is replaced by fibrous tissue containing large numbers of osteoclasts; this resembles the pathological picture of *osteitis fibrosa* (*von Recklinghausen's disease*). When large doses of parathyroid hormone are given to animals, changes in the bones are profound. Within a few hours many osteoblasts die, although the majority change into fibroblasta or osteoclasts or become phagocytic. There is widespread necrosis of the elements of the bone marrow and, at least in certain species, of the osteocytes. The calcium-containing trabeculae are rapidly resorbed and replaced by fibrous tissue. Salts of calcium have not been demonstrated within the osteoclasts, but have been found extracellularly as well as in the macrophages of the marrow. Recovery occurs when large numbers of osteoblasts develop from fibroblasts, and new bone, formed intramembranously, replaces the fibrous tissue (Fig. 121).

In long-standing deficiency of calcium and of vitamin D, especially when aggravated by pregnancy, the bones of adults contain much uncalcified osteoid tissue, and their mineral content is greatly diminished—a condition known as *osteomalacia* (*adult rickets*). The diminution in calcium content is due to failure of calcification of new bone formed in the turnover of this tissue, rather than to simple decalcification of previously calcified bone. In osteomalacia, and in the form of rickets induced experimentally by deprivation of calcium, the parathyroid glands are enlarged; they are much less affected in the rickets associated with deficiency of phosphate.

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JOINTS AND SYNOVIAL MEMBRANES

Bones are joined to one another by connective tissue structures which permit varying degrees of movement between the adjoining bones. Such structures are

casionally contain one or two vacuoles. There are no vacuoles within them which stain with neutral red. Mitochondria and a Golgi net have been demonstrated in them.

Folds of the synovial membrane may be either temporary formations which depend on the position of the joint, or they may form permanent *villi* which project into the joint cavity. Some of these villi have a broad base and a rather short stalk, while others may be quite thin and long. The larger folds contain blood vessels, lymphatics, and occasionally lobules of adipose tissue. There is an increase in the size and number of the villi with age. New islets of cartilage are formed in them, mainly by metaplasia of the synovial fibroblasts.

Blood vessels probably do not lie free on the surface of the synovial membrane. There are two plexuses of lymphatics, as a rule, within the synovial membranes, a superficial and a deep plexus The nerves which accompany the blood vessels end in the layer beneath the surface in terminal arborizations or end bulbs or plates. Pacinian corpuscles are always present.

When injured, the synovial membrane reacts as any other connective tissue by the formation of granulation tissue, and after some weeks may be completely regenerated. The synovial fluid is normally small in amount and seems to be a dialysate of blood to which have been added small amounts of mucin and a very few cells, chiefly lymphocytes, monocytes and macrophages.

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quently contain vessels. In other cases the two layers appear fused, or the synovial layer may rest directly on muscle or fatty tissue or periosteum. It has been suggested that the synovia be classified according to the tissues on which they lie, that is, loose connective, dense fibrous, or adipose tissue.

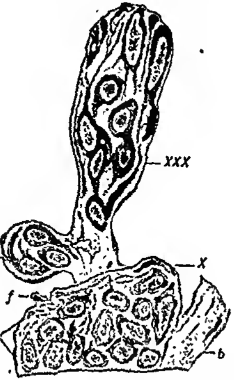

Fig. 123. End of a branching villus: *X*, Thicker portion of the villus, where many of the cells are of epithelioid appearance and their processes (*a*) are more superficial than the cell bodies; *b*, less cellular connective tissue cord; *XX* and *XXX* are small, secondary villi with branching cells; *f*, wartlike protuberance of the surface containing a cell process. After Hammar

Synovial membranes which rest on loose connective tissue usually cover those parts of the joints which are not subjected to strain or pressure. As a rule they have a definite surface layer, separated from the underlying tissue of the joint by loose connective tissue. The surface layer consists of collagenous fibers interspersed with fibroblasts whose processes may extend for long distances, although sometimes the cells are rounded. The fibroblasts do not form a complete covering for the interstitial collagenous substance; those cells on the surface have unusually long processes. The collagenous fibers are either irregularly arranged or they may be oriented along the main lines of stress. In addition to the fibroblasts, there are a few macrophages, leukocytes, and lymphoid wandering cells. In addition to blood vessels the loose connective tissue contains many *lymphatics.*

The fibrous synovial membrane covers the interarticular ligaments and tendons and lines those parts of the joints which are subject to strain. It consists of dense connective tissue; the surface zone is slightly more cellular than the rest. Some of the fibroblasts have capsules. When unusual pressure is applied to the synovial membrane, fibrocartilage develops.

The adipose type of synovial membrane covers the fat pads which project into the joint cavities. The synovial membrane in this case usually consists of a single layer of cells resting on a thin layer of connective tissue.

Fig. 124. Free surface of a thin synovial fold from which villi protrude: *v*, Small budlike villus without cells; *b*, superficial connective tissue, which is not covered by cells at b^1; *e*, cells arranged in an epithelial-like fashion, from which processes (*a*) arise. After Hammar.

The fibroblasts of the synovial membrane rarely show mitoses. They may oc-

Chapter VIII

MUSCULAR TISSUE

THE muscular tissue performs mechanical work by contracting, that is, by a shortening and thickening of its constituents. The contracting muscle cells regulate the position and movements of the various parts of the body with respect to one another. In the hollow viscera, ducts, and blood vascular system, the muscles propel the body liquids and excretions from place to place. Muscle cells are always elongated in the direction of the contraction and are usually grouped into bundles which sometimes reach a considerable length.

The vertebrates have two distinct types of muscle: *smooth muscle* and *striated muscle*, connected by many intermediate forms. As a rule, smooth muscles contract independently of voluntary control while the striated muscles are subject to voluntary control. *Cardiac muscle*, although striated, is involuntary and contracts automatically and rhythmically.

SMOOTH MUSCULAR TISSUE

Smooth muscle shows a very close relationship to the ordinary connective tissue and is found primarily in the internal organs. In man it forms the contractile portions of the wall of the digestive tract from the middle of the esophagus to the internal sphincter of the anus, of the ducts of the glands connected with the intestine, of the respiratory passages from the trachea to the alveolar ducts, and of the urinary and genital ducts. The walls of the arteries and veins and some of the larger lymphatics also consist to a considerable extent of smooth muscle. It is scattered in varying amounts in the connective tissue of the skin, in the capsule and trabeculae of the spleen, and in the connective tissue of certain sensory organs as the eye. Peculiar smooth muscle cells are often closely connected with the epithelial tissue of the dermal and salivary glands (see p. 291).

Smooth Muscle Cells or Fibers. When a bit of fresh smooth muscle is examined under the microscope, the muscle cells (i.e., fibers) appear as long, spindle-shaped bodies which are thickened in the middle and become narrow toward their pointed ends (Fig. 125). Occasionally, the cells are branched or star-shaped. As they adjust themselves to the spaces available to them, their external form depends to some degree upon the surrounding elements.

The smooth cells may reach a length of 0.5 mm. in the pregnant human uterus, while their average length in the intestine of man is 0.2 mm. with a thickness of 6 μ. The smallest smooth muscle cells occur in the small blood vessels where they are 15 to 20 μ in length.

The nucleus, as seen in cross sections, is slightly eccentric and occupies the middle, widest portion of the cell body. It is elongated in the long axis of the cell and has an oval or cylindrical form with pointed or rounded ends. It contains several nucleoli and a pale chromatin net which lies along the internal surface of the nuclear membrane. During the contraction of the cell the nucleus becomes folded on its lateral surface or markedly twisted. In general, the size of the nucleus increases with the size of the cell. Close to the nu-

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associated that sarcoplasm cannot be noticed between them. Mitochondria, a Golgi net, and sometimes glycogen granules have been described in the sarcoplasm.

Contact of Smooth Muscle Cells with One Another. In many places in the body, but particularly in the skin, smooth muscle fibers are scattered singly or in small groups in the ordinary connective tissue. Here they are closely welded to the collagenous bundles and are often surrounded by thin elastic fibers. During contraction they throw the tissue into fine folds and wrinkles; this can be well seen in the skin of the mammary papillae or the scrotum. Sometimes several parallel fibers unite to form a small cylindrical bundle (that is, a muscle) whose ends are covered by elastic fibers. An example is the smooth muscles connected with the hairs.

In other cases the smooth muscle fibers are arranged parallel to one another in one plane where they form a layer of varying thickness, as in small arteries. Here, because of the small lumen, each fiber has to bend sharply in order to surround the vessel. In the walls of certain large hollow organs, as the intestine, bladder, and uterus, the smooth muscle cells are arranged in layers or bundles. The *direction of the fibers is the same in each* layer, while it varies in different layers or bundles. Thus, in the intestine the internal layer of the muscularis externa consists of circularly arranged fibers forming a close spiral, while the external layer is composed of longitudinally arranged fibers forming a long spiral (Carey). (See Fig. 127 and Chapter XIX.)

The cells are so arranged that the thick middle portion of one cell is opposite the thin ends of adjacent cells. Consequently, in cross sections through a smooth muscle bundle, some of the cells have nuclei in the plane of the section and some do not.

The connective tissue fibers outside of the muscle cells continue into the spaces between them and bind them into the bundles. Between the thicker bundles and layers of smooth muscles, loose connective tissue is present in small amounts. It contains fibroblasts and wandering cells, collagenous and elastic fibers, a thick network of blood vessels and nerves. But

Fig. 127. Longitudinal section of a part of the muscularis externa of the intestine of a dog; external longitudinal layer (below), cross section of internal layer (above); and *c*, blood capillaries. 530 ×. (A.A.M.)

connective tissue cells do not occur in the narrow slitlike spaces between the individual smooth muscle cells; here there are only a few thin, collagenous bundles and dense networks of reticular and elastic fibers.

The reticular fibers branch irregularly and pass longitudinally and transversely

cleus, in a small indentation of its membrane, is a diplosome without an attraction sphere.

Smooth muscle cells do not possess a distinct membrane which corresponds with the sarcolemma of the striated muscles (p. 000). The cytoplasm of smooth muscle cells in the living condition and sometimes after fixation and staining appears to be quite homogeneous. But some threads, the *myofibrils,* can always be made visible by maceration in nitric or trichloracetic acid. After this treatment the individual fibrils separate and become visible as parallel threads running the length of the cell (Fig. 126).

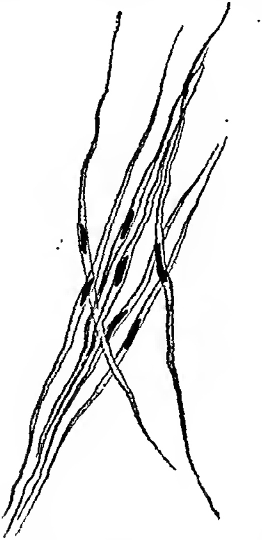


Fig. 125. Isolated smooth muscle cells from the wall of the stomach of a cat. 220 ×. (A.A.M.)

The fibrils are quite homogeneous and differ from those of the striated muscle fibers in that they do not have the alternating sections characteristic of the latter. Accordingly, the muscle cells which contain homogeneous fibrils are called ***smooth muscle cells.*** The fibrils are doubly refractile. The *border* or *external fibrils* are coarse and thick and stain deeply; they are located inside the peripheral layers of the cell. In a cross section through a smooth muscle cell, they appear as sharply stained dots near the edge of the cytoplasm. The internal fibrils are thinner, measuring 0.3 μ. The number of fibrils in the smooth muscle cell varies and in some cases the border fibrils may be very few in number or absent.

Fig. 126. Smooth muscle cells of the intestine of *Triton vulgaris;* the myofibrils are very distinct Biondi stain. 1430 ×. After Levi.

It is generally believed that the fibrils represent the contractile elements of the smooth muscle cell. The *myosin* of smooth muscle is similar to that of striated muscle (Fischer). The substance between the fibrils is called *sarcoplasm;* it usually accumulates in small amounts at both ends of the nucleus. Most of the cell body is occupied by fibrils which are so closely

myoblasts which lie against the endothelium, come in contact with one another, and a continuous layer of smooth muscle is produced.

The reticular fibers between the muscle cells are probably produced by the same cells which become muscle fibers. If this is true, then the developing smooth muscle cells function as both myoblasts and fibroblasts. The smooth muscle elements (myo-epithelial cells, p. 291) in certain glands arise from the same epithelium from which the glandular elements arise.

According to one author, some of the new smooth muscle cells which develop in the uterus during pregnancy arise from the undifferentiated connective tissue cells already present in this tissue as well as from lymphocytes which wander into the myometrium in the early stages of gestation. This conclusion is denied by other investigators who studied the changes in the virgin rabbit uterus after injection of the female sex hormone. They concluded that the hypertrophy of the smooth muscle in these experiments was due to mitotic proliferation of the smooth muscle cells.

In the vicinity of injured regions in the walls of the intestine or stomach, mitosis has been observed in the smooth muscle cells. But this capacity for regeneration is small and, as a rule, great defects in the smooth muscle tissue heal by scar formation. Mitoses have been described in the muscle cells of the nonpregnant human uterus. Whether smooth muscle cells in the adult organism may be formed anew from fibroblasts has not been established; it is practically certain that they may develop from the perivascular embryonic cells of the adult (p. 61).

STRIATED MUSCULAR TISSUE

The Muscle Fibers. The muscles attached to the skeleton of mammals consist of striated muscular tissue. In a teased preparation of fresh striated muscle, the tissue appears to consist of long cylindrical *muscle fibers*. These are large multinucleated cells. As a result of teasing, only torn and broken sections of the fibers are seen and the normal ends of the fibers are seldom found. However, when their undamaged ends are seen, the gradual tapering of the fibers toward a point is clearly visible. In other cases the end of the fiber appears rounded or notched; such appearances are particularly frequent at the union of the muscle with a tendon.

Although the fibers are usually very close to one another, they are entirely independent. Occasional anastomoses have been described between them. In nontapering muscles like the sartorius the fibers apparently continue without interruption through the entire muscle so that their length is equal to that of the muscle (Lockhart and Brandt). It is generally believed that in most muscles the fibers are usually shorter than the *muscle;* in this case one end may be connected with a tendon while the other end terminates among other fibers, or both ends may be

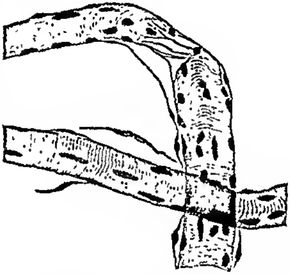


Fig. 130. Two striated muscle fibers of man, in a teased preparation, with stained nuclei. The upper fiber is crushed in its middle and here the sarcolemma is seen. Between the fibers are several spindle shaped connective tissue cells. 250 ×. (A.A.M.)

free in the muscle. This statement should be subjected to further investigation. The thickness of the fibers fluctuates from 10 to 100 μ or more; apparently it depends not on the length of the fiber, but on the type of animal and the particular muscle. In a given animal the more primitive muscles, as those of the eye, are thinner. Fibers of varying caliber may be found in the same muscle. The thickness of the fibers increases with the age of the organism as well as under the influence of strenuous muscular activity.

Even in teased preparations of fresh

between the bodies of the smooth muscle cells. They can be stained with Mallory's aniline blue method and still more sharply with the silver impregnation methods. They form a regular system of supporting and binding material which forms a *sheath* about each muscle cell (Fig. 128). A characteristic of smooth muscle all over the body is the intimate association it bears with elastic fibers. This is so extensive that some authors consider them as forming a "myo-elastic" tissue. These elastic fibers are continuous with those of the surrounding loose connective tissue.

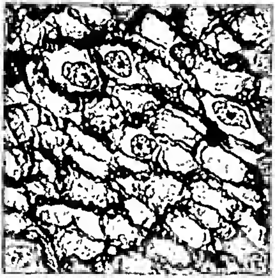
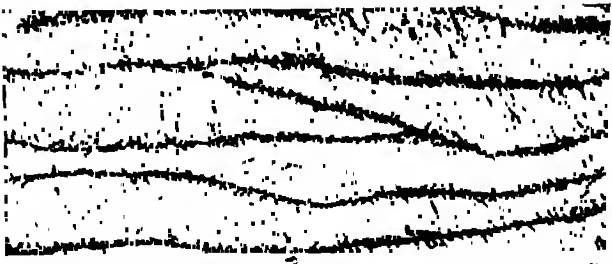


Fig. 128. Cross section through smooth muscle from human intestine, stained with hematoxylin and the Bielschowsky silver method for reticular fibers. The latter form continuous networks about each of the muscle cells. Note how few of the cells have their nuclei in the plane of the section. 1875 ×. *Drawn by Miss E. Bohlman.*

In smooth muscles, the pull of each contracting cell is first transmitted to the surrounding sheath of reticular fibers which continue directly into those of the surrounding connective tissue. This arrangement permits the force of the contraction of the entire layer of the smooth muscle to be uniformly transmitted to the surrounding parts, as in the narrowing of the lumen of blood vessels or in peristalsis of the intestine.

Histogenesis and Regeneration of Smooth Muscle. Smooth muscle cells arise from the mesenchyme. In those places where a layer of smooth muscle will later develop, the mesenchymal cells begin to stretch out, the nuclei become elongated, and thick fibrils appear in the cytoplasm. They probably do not develop from the mitochondria. The thick myofibrils, at least in the beginning, apparently run continuously through a whole series of cells; later they split into thin myofibrils. In blood vessels, which at first consist only of endothelium, the muscle cells are formed as follows: Mobile mesenchyme cells become arranged at regular intervals along the outside of the endothelial tube. They stretch out transversely, multiply by mitosis and then produce myofibrils within their cytoplasm. Then the edges of these



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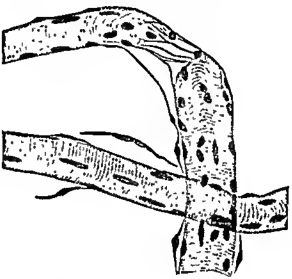

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Even in teased preparations of fresh

muscle, the complex and peculiar structure of the striated fibers is seen readily. They are covered with a thin (1 μ) structureless membrane, the *sarcolemma*. In uninjured regions it is invisible as it closely adjoins the substance of the fibers. But in those places where the fiber has been torn or crushed, the sarcolemma appears as a transparent film (Fig. 130).

The freshly teased fiber is usually slightly yellow and striated in both the longitudinal and transverse directions. The distinct and regular cross striation is obvious in the living cell. From its presence the tissue has received the name of *striated* muscle. These striations depend with the large size of the fibers (Fig. 130).

The chief solid mass of the muscle fibers consists of various albuminous substances of which the most *important* are *myosin* and *myogen*. Striated muscle contains more myogen and less myosin and one tenth as much nucleoprotein as smooth muscle. The fibers also contain carbohydrates, fats, lipoids, and a pigment *myoglobin* which is closely related to hemoglobin. Cytochrome, which consists of several hemochromogens, is also present. The fibers contain various metabolic intermediaries such as lactic acid, creatin-phosphate, hexosephosphate.

Sarcolemma. The sarcolemma is a thin (1 μ), structureless membrane, which completely invests the fiber and fol-

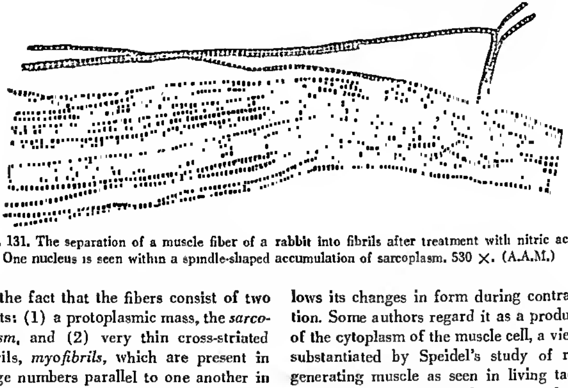

Fig. 131. The separation of a muscle fiber of a rabbit into fibrils after treatment with nitric acid. One nucleus is seen within a spindle-shaped accumulation of sarcoplasm. 530 ×. (A.A.M.)

on the fact that the fibers consist of two parts: (1) a protoplasmic mass, the *sarcoplasm*, and (2) very thin cross-striated fibrils, *myofibrils*, which are present in large numbers parallel to one another in the sarcoplasm.

The transverse striation is believed to depend on the fact that each myofibril consists of *disks* or sections which alternate regularly along its length. The corresponding disks of adjacent fibrils are usually arranged at the same level in the fiber and cause the regular, transverse striation of the fiber.

The nuclei are seen in a fresh preparation of striated fibers, particularly after staining. Their great number corresponds

lows its changes in form during contraction. Some authors regard it as a product of the cytoplasm of the muscle cell, a view substantiated by Speidel's study of regenerating muscle as seen in living tadpoles, while others consider it a product of the connective tissue surrounding the muscle fibers. Several authors have described a fibrillar structure in it, which they compare with the basement membrane. The chemical reactions of the sarcolemma differ, however, from those of collagen and elastin.

Myofibrils. In longitudinal sections through muscle fibers or in preparations where they have separated into individual fibrils, the latter appear as long, parallel

threads (Fig. 131), which do not branch. The thickness of the fibrils fluctuates, but they are not larger than 1 to 2 μ in ordinary preparations, and some authors have given 0.2 μ as the lower limit.

Hall, Jakus and Schmitt found that in

lines for indefinite lengths in the fibrils.

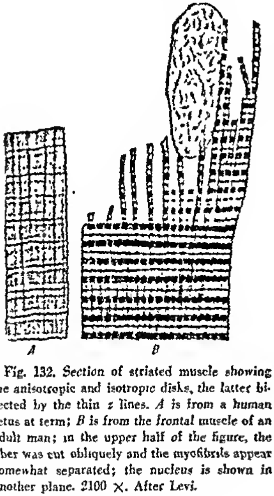

Fig. 132. *Section of striated muscle showing* the anisotropic and isotropic disks, *the latter* bisected by the thin *z* lines. *A* is from a human fetus at term; *B* is from the *frontal* muscle of an adult man; in the upper half of the figure, the fiber was cut obliquely and the myofibrils appear somewhat separated; the nucleus is shown in another plane. 2100 ×. After Levi.

Myofibrils are distributed evenly or in compact bundles in the cytoplasm, the spaces between the fibrils being occupied by sarcoplasm. In cross section the separate fibrils appear as fine dots. When the fibrils are packed in bundles, the latter are separated by sarcoplasm.

Structure of the Myofibrils. The appearance of the striated muscle fiber depends on whether it is at rest, contracted, or passively stretched. The myofibril is *composed* of two main types of substance which alternate regularly along its length. They are short cylinders and are called

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doubly refractile, or *anisotropic*, in living cells as well as after fixation, had best be designated A disk (also called Q). It is undecided what causes the anisotropism of the A disks.

Alternating with the A disks are *isotropic* (singly refractile) or I disks (I in German). With the usual stains the I disks remain colorless. In resting fibrils the A

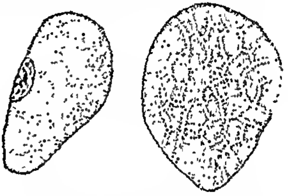

Fig. 133. Cross section of two striated muscle fibers of a rabbit. In *A*, a uniform distribution of fibrils, in *B*, Cohnheim's fields. 1000 ×. Redrawn after Szymonowicz.

and I disks are approximately the same height. When the fibers are passively stretched the I disk appears taller than the A disk.

There are other disks in the myofibril; the most important of these is the Z or *intermediate disk*, which is doubly refractile and occupies the middle of the I disk. But unlike the A and the I disks, the Z disk is not confined to the myofibril but passes through the entire diameter of the fiber. During contraction the center of the A disk appears paler than the rest and is called Hensen's disk, within which a thin middle stripe indicated by the letter M

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Sarcolemma. The sarcolemma is a thin (1 μ), structureless membrane, which completely invests the fiber and follows its changes in form during contraction. Some authors regard it as a product of the cytoplasm of the muscle cell, a view substantiated by Speidel's study of regenerating muscle as seen in living tadpoles, while others consider it a product of the connective tissue surrounding the muscle fibers. Several authors have described a fibrillar structure in it, which they compare with the basement membrane. The chemical reactions of the sarcolemma differ, however, from those of collagen and elastin.

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Fig. 131. The separation of a muscle fiber of a rabbit into fibrils after treatment with citric acid. One nucleus is seen within a spindle-shaped accumulation of sarcoplasm. 530 ×. (A.A.M.)

to the type of muscle, one or the other type of fibers predominates.

Organoids and Inclusions. The sarcoplasm of many striated muscle fibers contains fat droplets, pigment and lipoid granules, and glycogen. In addition to these inclusions, the sarcoplasm contains mitochondria and particles called sarcosomes. The latter occur in both the I and the A disks, and their exact nature is undecided. As the sarcosomes and mitochondria have been demonstrated in preparations made with different technics, the question is unsettled as to possible interrelationships between them. The fat inclusions diminish or disappear in starvation. In fixed preparations glycogen appears as granules of irregular shape and size between the myofibrils. The sarcoplasm also contains a small Golgi net, frequently at each pole of the nucleus.

Nucleus. The nuclei of striated muscle fibers are rounded, oval or elongated in the direction of the long axis of the fiber. Their size varies considerably. Their number fluctuates within wide limits as it depends on the length of the fiber. The nuclei are always numerous, and in fibers several centimeters long they number several hundred.

The position of the nucleus varies according to the species of animal and the type of muscle. In the muscles of the lower vertebrates, and in many of the red muscles of mammals, the nuclei are scattered throughout the entire fiber. In the great majority of striated muscles of mammals, the nuclei are in the layer of sarcoplasm immediately beneath the sarcolemma.

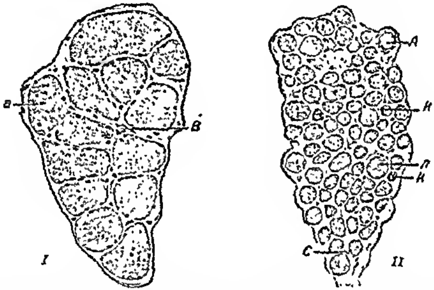


Fig. 135. *Cross section of muscle bundles from two muscles of the same man treated in the same way. I,* Gastrocnemius muscle; *a,* a fiber with central nuclei; *B,* blood vessel. *II,* An ocular muscle; *n,* normal fiber with Cohnheim's fields; *k,* dense portion of a fiber; *A,* artery; *C,* capillary. Both figures 110 ×. After Schaffer.

The Union of Striated Muscle Fibers with One Another to Form Muscles. Muscles are formed of parallel muscle fibers held together by connective tissue. The arrangement of muscle fibers within the muscle is clearly seen in cross sections and is similar to the structure of tendons. As the collagenous bundles in tendons, so the muscle fibers here, combine to form the *primary bundles;* several primary bundles combine to form *secondary bundles; tertiary bundles* are formed by the secondary ones, etc. Large bundles and layers of interstitial connective tissue at the periphery of the muscles, the *epimysium,* project into the spaces be-

may be seen. The N disk may be seen at times within the I disk between the Z and A disks.

The electron micrographs of Hall, Jakus and Schmitt show that the Z disks are not collagenous (as some had believed) and that the I as well as the A disks consist of myosin filaments. These are apparently the contractile units.

The amount of substance included between two Z disks is called a *sarcomere*. This has been considered to be the structural and functional muscular unit (Fig. 134).

According to studies made with the older histochemical methods and with the aid of microincineration (Scott) it is believed that there is a concentration of potassium in the A disks. Gersh claims that potassium, phosphate, and carbonate are uniformly distributed in the muscle cells. Potassium is thirty times, and phosphate at least eight times as concentrated in the muscle cells as in the interstitial tissue.

In the invertebrates, especially in insects, many other disks may be seen in striated muscle.

Sarcoplasm. The cytoplasm of the muscle fiber which fills the spaces between the myofibrils is called sarcoplasm. The sarcoplasm accumulates constantly around the nuclei, especially at their ends. It is also present in small islands about the terminal endings of the motor nerves.

Variations in the Amount of Sarcoplasm. It is customary to distinguish striated muscle fibers rich in sarcoplasm from those poor in sarcoplasm. In certain vertebrates, as the rabbit, one can even recognize two types of muscles with the naked eye; one appears red and the other white. Under the microscope the fibers of the first type are seen to be rich, and those of the second type poor, in sarcoplasm. In the pale cells the fibrils are very small and regularly arranged. In the fibers of red muscles the longitudinal striation is more prominent and the transverse striation is somewhat irregular. The red fibers contain many fat granules and have a "muddy" appearance. The nuclei of the red fibers occupy a more central position in the fiber and are rarely found at the periphery. As a rule, the red granular fibers in the lower vertebrates are confined to the most important muscles, while in the higher animals only the least active muscles are white. The red pigment is usually found in those muscles which contract repeatedly and over long periods of time. The "twitch" is longer in red muscles and they are more easily "tetanized." Those muscles which contract slowly are red, but all red muscles do not contract slowly. The pigments which give these fibers their red color are probably muscle hemoglobin and cytochrome.

In the rabbit each type of fiber is generally gathered into separate muscles which show certain physiologic differences. In the majority of animals including man, both types of fibers enter into the composition of all the muscles; according

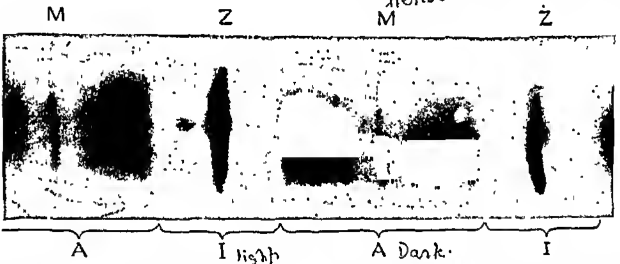


Fig. 134. Electron micrograph of myofibril from leg muscle of a frog. 32,000 ×. Courtesy of C. Hall, M. Jakus, and F. Schmitt.

the collagenous fibrils of the tendon. The transverse striation of the myofibrils disappears at such places. This conception has been denied by other investigators who claim that the collagenous fibrils of the tendon only *appear* to continue into the myofibrils. The problem obviously requires experimental investigation; the method of microdissection might be of distinct aid; certainly, the continued study of the musculo-tendon junction by purely morphological methods has led to an impasse of diametrically opposed views. Observations on living regenerating muscle in tadpoles indicate that the myofibrils continue directly into the tendon fibrils and that in development a part of the tendon may arise from the myoblast (Speidel).

Blood Vessels and Nerves. The blood vessels of skeletal muscle tissue (particularly red muscles) are very abundant, capillaries which directly enlace separate fibers form a dense network with meshes stretched along the length of the fiber (Fig. 138). The short sides of these meshes sometimes appear as swollen spindles.

Lymphatics have been found in certain muscles within the layers of the perimysium and around the blood vessels.

The main nerve supply of the skeletal muscles is from the myelinated cerebrospinal afferent and efferent nerves.

Histogenesis of the Striated Muscle Tissue. The striated muscular tissue arises in vertebrates from the mesoderm and in particular from its primitive segments. The lower part of the medial layer of each segment forms a mass of embryonic connective tissue, the *sclerotome*, while the lateral layer of the segment produces the *skin plate*. The remaining part of the medial layer of the segment, the *myotome*, represents the primordium of the dorsal as well as the ventral musculature of the body. It is possible that cell masses growing out of the myotome take part in the formation of musculature of the appendages; but here, as in the development of head muscles, the participation is not direct and the laying down of the muscular tissue cannot be separated distinctly from the mesenchyme.

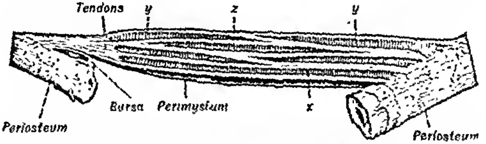


Fig. 137. Diagram showing connections between a muscle and the bones to which it is attached. Muscle fiber, *x*, begins and ends in tendons attached to the bones; *y*, terminates at one end in the muscle; *z*, terminates at both ends in the muscle. After Braus.

Fig. 138. Photomicrograph of carmine-gelatin injection of blood vessels in striated muscle of cat. Note arrangement of vessels parallel to muscle fibers and right-angle branchings. 125 ×.

Those cells which give rise to the muscle tissue are called *myoblasts*. Within the myotome they are regular and cylindrical, but very soon they stretch, become spindle shaped and arrange themselves into parallel bundles. At the same time they multiply rapidly by mitosis.

There are several explanations of how the large,

tween the bundles of muscular fibers as the *perimysium*. They consist of irregularly arranged collagenous, reticular and elastic fibers, and many varieties of connective tissue cells including fat cells. These thick layers branch and send thin layers between the smaller bundles (Fig. 136).

Between the separate muscle fibers inside the primary muscle bundles the *endomysium* consists, as in smooth muscles, of thin fibrous networks which form capsules for the fibers. The endomysium also contains fibroblasts and fixed macrophages. The latter play an important rôle as phagocytes in inflammation of the muscle.

The number of elastic fibers in the interstitial connective tissue varies with the type of muscle and is probably closely connected with its functional peculiarities. They are extremely abundant in the eye muscles and in muscles which attach to soft parts (muscles of the tongue and face).

Where a muscle is attached to a tendon there is a very close union of the muscle fibers with the collagenous bundles of the tendon. The collagenous bundles of the perimysium pass directly over into those of the tendon; the sarcolemma covering the rounded, cone-shaped ends of the muscle fiber is fused with the ends of the collagenous bundles. The cone-shaped ends of the muscle fiber fit into grooves in the tendon. The connection of the sarcolemma with the collagenous bundles is much more rigid than with the substance of the muscle fiber itself; for if a fresh muscle is put into hot water, the contents of the muscle fiber at the ends separate from the sarcolemma.

Several authors have claimed that, besides the union of the sarcolemma with the tendon, there is a direct continuation of the myofibrils of the muscle fiber into

Fig. 136 Cross section through the sartorius muscle of man showing the subdivision into bundles of various sizes by connective tissue. 4 ×. Photograph by Mütler, from Heidenhain.

Z disks. Following this the thin regions of the threads between the thickenings develop into isotropic disks. At first the myofibrils are separated from one another; they connect up secondarily through continuous membranes, the Z disks.

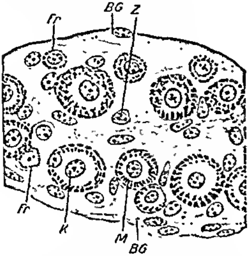


Fig. 141. Cross section through an embryonic muscle bundle of a sheep. *BG*, Cells of the primordium of the perimysium; *fr*, muscle fibers in cross section with a single layer of primitive fibrils; *K*, nucleus in the axial sarcoplasm; *M*, primitive muscle fibers; Z, connective tissue cell. 740 ×. After Schaffer.

The continued increase in the number of myofibrils is by longitudinal splitting (Fig. 140).

Although many investigators believed that mitochondria give rise to myofibrils, this idea is probably incorrect. The mitochondria remain in the sarcoplasm between the fibrillar columns and accumulate around the nuclei.

The fibrils occupy the peripheral part of the fiber *while the axial portion is occupied by un*differentiated cytoplasm with short mitochondria, and nuclei which continue to divide (Fig. 141).

The further development of the muscular tissue consists of a combination of several phenomena. First, the separate fibers increase in thickness and length. Then their number increases, at first through the transformation of new myoblasts, and later by a longitudinal splitting of already existing fibers.

According to one author, the increase in number of fibers in the sartorius muscle stops when the human embryo is 130 to 170 mm. long. The future growth of the muscle depends only on the continued increase in the size of the fibers already present. As the growth of the different fibers in the same muscle does not stop simultaneously, fibers of various thicknesses result. Other authors, on the contrary, find both the splitting of fibers and the continued new formation of fibers from undifferentiated elements in newborn mammals.

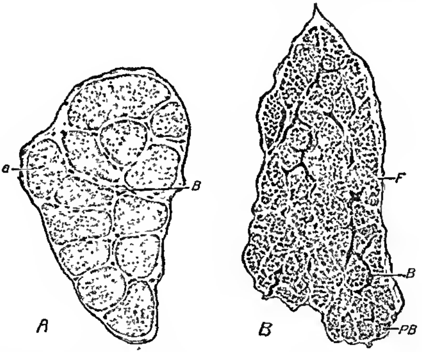


Fig. 142. Cross section through the gastrocnemius muscle of an adult (*A*) and of a six-month fetus (*B*). Notice the difference in thickness of the fibers. *a*, A fiber with central nuclei; *B*, blood vessel; *PB*, primary bundle, the number of whose fibers corresponds to those in Figure *A*; *F*, connective tissue. 110 ×. After Schaffer.

multinucleated, skeletal muscle fibers arise from the myoblasts: (1) Each muscular fiber is a syncytium resulting from secondary fusion of many separate cells. (2) Each myoblast grows markedly in length and the rapid multiplication of the nuclei by mitosis (and perhaps later by amitosis) is not accompanied by division of protoplasm so that a multinuclear cell is produced. (3) Both methods of development occur. A final decision of this question must be left to future investigations.

In the young, spindle-shaped myoblasts, thin threads appear, which in the beginning are quite homogeneous, but which later acquire thickenings at equal distances along their entire length; these are the precursors of the A disks. After this a second series of thickenings appears, halfway between the first; these are the forerunners of the

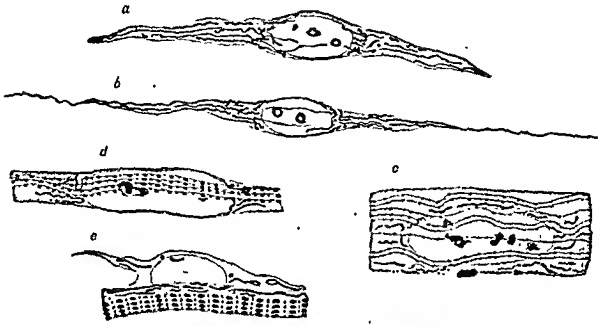


Fig. 139. The development of myofibrils within the myoblasts of a chick embryo: *a*, Myoblast of sixty-hour embryo; *b*, myoblast of seventy-six-hour embryo; *c*, middle portion of the myoblast with nucleus from a one-hundred-twenty-four-hour embryo showing the first appearance of segmentation of the myofibrils; *d* and *e*, later stages. Redrawn after Duesberg.

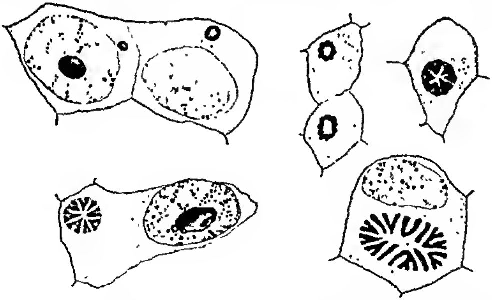


Fig. 140. Cross section of developing muscle fibers of a trout embryo. Within the protoplasm of the fiber, one thick fibril is seen in cross section as a black spot. In the following stages it splits into a larger and larger number of radially arranged fibrils. 3800 ×. Redrawn after Heidenhain.

The sarcolemma appears at the surface of the embryonic muscle fibers at comparatively late stages.

The nuclei, during the gradual growth of the muscle fiber, increase in number by mitosis, and in later stages, perhaps, by amitosis. In mammals the nuclei in the beginning are located in the center, while the fibrils occupy the periphery of the fiber. During later stages the nuclei move toward the periphery, so that the central parts become occupied by fibrillar columns (Fig. 142).

The ability to contract in response to direct irritation begins in the embryonic muscular elements at about the time or shortly before the first myofibrils arise in their protoplasm. It has been suggested that the myofibrils develop as the result of the tension of the developing, contracting muscle. This contractility, at first slight and slow, gradually increases with the increase in number of myofibrils and their arrangement in bundles. The appearance of voluntary movements is closely connected with the development of the nervous motor tracts which lead from the spinal cord to the myotomes.

During the embryonic histogenesis of the striated muscle tissue, degenerative processes accompany the phenomena of progressive character. These sometimes include a considerable portion of the muscle fibers and may lead to their complete destruction.

Regeneration of Striated Muscular Tissue. During intensive activity, the skeletal muscles increase in volume; this depends on the enlargement of the already existing fibers through an increase in the sarcoplasm and not of the fibrils.

The regenerative capacity of the striated muscular tissue of vertebrates, particularly of the higher ones, is usually insignificant and does not always lead to the formation of functioning fibers. After destruction of muscle fibers, regeneration always starts from the fibers already present. The most successful regeneration takes place in those cases when only the contractile substance is destroyed while the sarcolemma and the peripheral layer of sarcoplasm with the nuclei are intact. The nuclei with the surrounding sarcoplasm remain alive and are the source of regeneration—these are now separate cells called *sarcoblasts* or *myoblasts*. The ends of the fibers become thicker and grow out toward the place of injury as simple or branched muscular buds. The sarcoblasts hypertrophy, multiply, digest the degenerating fibers and later fuse in groups. Inside the old sarcolemma they form new fibers in which striated fibrils appear.

During such a regenerative process, the sarcoblasts or myoblasts can be easily distinguished in vitally stained animals from the macrophages which have penetrated the fiber (see Speidel, 1938).

It has been claimed that muscle cells turn into macrophages under the influence of cholin in tissue cultures.

A large defect in the muscular tissue is replaced by a connective tissue scar.

The maintenance of a connection with the motor nerve fibers is necessary for the normal existence of the skeletal muscular tissue as well as for its successful regeneration This "trophic" influence of nerve fibers on striated muscular elements becomes apparent even when a small piece of muscular tissue is transplanted into some thick nerve trunk, as the sciatic, of the same animal. In this case a rather energetic, although temporary, regeneration of the transplanted muscle fibers is observed. For details of the atrophy of muscle after section of the nerves see Tower.

CARDIAC MUSCULAR TISSUE

The heart of all vertebrates is composed of a network of peculiar striated muscle fibers. It contracts rhythmically and automatically. Its structure differs in several respects from that of skeletal striated muscles.

In a section of mammalian cardiac muscle, parallel to the surface of the heart, one can see that the fibers form networks with narrow meshes stretched in one main direction. In cross section the fibers appear as rounded or irregular areas of various sizes. Free endings can be found in cardiac muscle fibers only in teased, macerated preparations taken from the region of the atrioventricular openings and from the papillary muscles.

The cardiac muscle fibers consist of: (1) nuclei; (2) myofibrils; (3) sarcoplasm; (4) a sarcolemma, according to some authors, and (5) intercalated disks.

Nuclei. The nuclei in cardiac muscle fibers, in contrast to those of striated muscle, are always arranged in the interior, usually in the axial part, of the fiber. They are scattered in the network of the fibers at various distances from one another. Their shape is oval and their internal structure shows nothing peculiarly

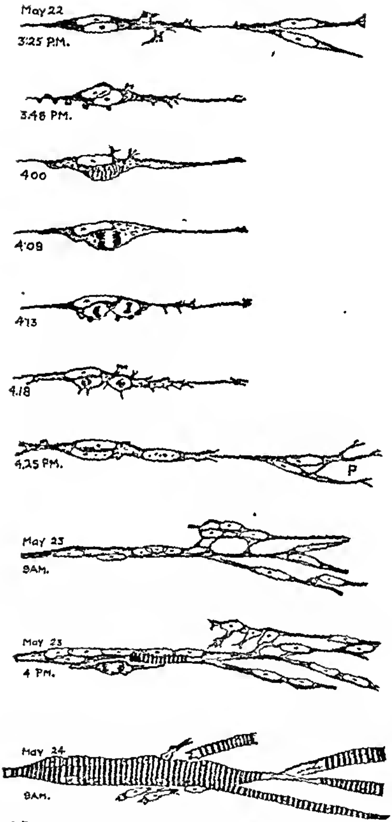


Fig. 143. The differentiation of myoblasts into cross-striated muscle fibers as seen in living, regenerating zone, following removal of the tip of a tadpole's tail. One of a pair of closely associated myoblasts was watched throughout its nuclear division. An anastomosing process (*P*) developed between the two myoblasts to the right during this time. The next day many nuclei were present and the mass was under increased traction in a longitudinal direction. At 4 P. M. the first faint cross striations were visible. The following day many cross striations were in evidence in all fibers. Redrawn after Speidel.

what less than that of a sarcomere; but like a sarcomere the intercalated disks are practically always bounded on both sides by the Z plates. Where the fibers branch, intercalated disks are found which are cone-shaped in sections (Fig. 145).

The substance of the intercalated disks stains sharply with various dyes. Under high magnification it appears to consist of vertical rods whose ends are directly fused with the myofibrils approaching the disk from both sides. The myofibrils are believed to pass uninterruptedly through the intercalated disks.

These areas consist of a number of branched processes which are separated from one another in the longitudinal direction by intercalated disks. The number of nuclei in each segment is rather constant for each type of animal; it is usually 1 to 2 in man and may be as great as 32 in the pig. It was therefore supposed that cardiac muscle is composed of independent muscle cells and that the intercalated disks are the cell boundaries But most authors do not believe that these disks are cell membranes. They point out that the fibrils pass uninterruptedly through the intercalated disks from one segment into the next. They have also shown that in each segment there is at least one place where it is not separated by an intercalated disk from the adjacent segment and that consequently both segments are connected by anastomoses.

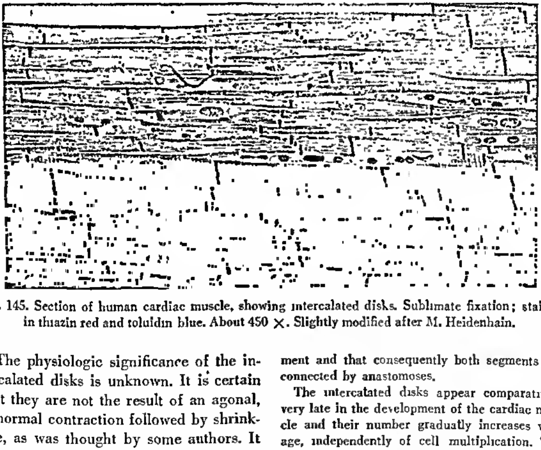


Fig. 145. Section of human cardiac muscle, showing intercalated disks. Sublimate fixation; stained in thiazin red and toluidin blue. About 450 ×. Slightly modified after M. Heidenhain.

The physiologic significance of the intercalated disks is unknown. It is certain that they are not the result of an agonal, abnormal contraction followed by shrinkage, as was thought by some authors. It is not probable that these disks represent areas where the cardiac fibers are able to grow in length, that is, where the new formation of sarcomeres is possible. According to another opinion, these areas act as fine, elastic interstitial tendons. The intercalated disks have also been considered to be altered Z disks which divide the network of cardiac muscle fibers into cell territories.

The intercalated disks appear comparatively very late in the development of the cardiac muscle and their number gradually increases with age, independently of cell multiplication. The disks are, therefore, a sign of secondary, incomplete division of the entire mass of cardiac muscle into separate territories or segments. According to most authors, cardiac muscle represents a syncytial, multinucleated mass of reticularly arranged, protoplasmic bars in which contractile *fibrils pass independently of cell territories.*

It has been clearly shown in tissue culture that two heart muscle cells, which are not completely separated by a distinct

different from that of the nuclei of skeletal muscle fibers (Fig. 144).

Myofibrils. The myofibrils are similar to those of ordinary striated fibers, and are composed of the same types of disks, A, I, Z, etc. Transversely they are connected by the continuations of the Z disks passing through the sarcoplasm.

The fibrils in the cardiac muscle pass uninterruptedly along the length of tho fiber so that their free ends are not seen. The cross-striated fibers here, too, are gathered into bundles or columns. The individual fibrils have been seen in living tissue cultures (Hogue, 1937).

Sarcoplasm. The sarcoplasm in the cardiac muscle fibers is usually rather abundant; due to this fact the longitudinal striation is well pronounced. In the nuclear areas it forms elongated accumulations which are devoid of fibrils; the swollen middle portion of these accumulations contains the nucleus and tapers gradually to the ends (Fig. 144). Mitochondria are scattered in the sarcoplasm. In thc spindle-shaped accumulations which surround the nuclei there are also fat droplets, a poorly developed Golgi net and pigment granules which increase with age.

Sarcolemma. The free surface of the cardiac muscle fibers is provided with a very thin sheath which is well seen in sections as a sharp line. It is not so easily isolated as the sarcolemma of striated skeletal muscle. It may be selectively stained with certain dyes and the Z disks are attached to it in the same way as to the sarcolemma of skeletal muscles (Fig. 144, *S*). The origin of this sheath is not clear; some authors regard it as a condensation of sarcoplasm, others as a product of the interstitial connective tissue. Well pronounced, interstitial connective tissue areas are found only in the cardiac muscle of adult mammals.

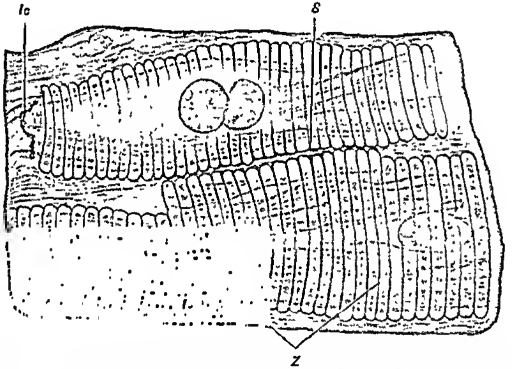


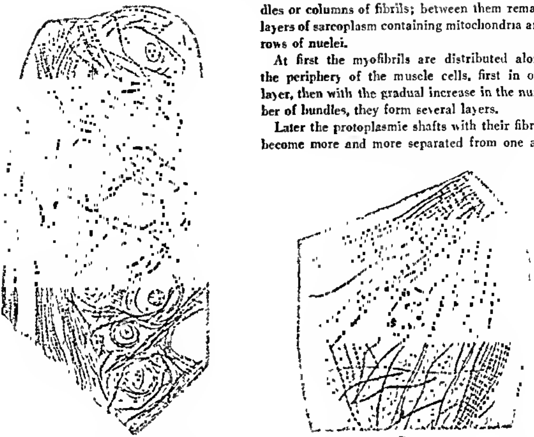
Fig 144. Section of human cardiac muscle fibers showing connection between the Z disks (Z) and the sarcolemma (*S*); *Ic*, intercalated disk. Mallory-Azan stain. 1400 ×. Drawn by Miss Agnes Nixon.

Intercalated Disks. For a long time the attention of investigators has been attracted by short lines or V-shaped stripes, called *intercalated disks*, which are oriented transversely to the long axis of the fibers. The height of the stripes is some-

dles or columns of fibrils; between them remain layers of sarcoplasm containing mitochondria and rows of nuclei.

At first the myofibrils are distributed along the periphery of the muscle cells, first in one layer, then with the gradual increase in the number of bundles, they form several layers.

Later the protoplasmic shafts with their fibrils become more and more separated from one an-



A B

Fig. 147. Cells of Purkinje in a papillary muscle of a sheep. *A*, Concentric arrangement of the myofibrils in the peripheral part of the cells. 475 ×. *B*, The myofibrils have the same A and Z disks as those of the typical cardiac muscle fibers. 1275 ×. After Levi

endothelial tube. These elements at first are a layer of cuboidal cells, loosely connected by intercellular bridges. Then this portion of the splanchnopleure thickens, becomes stratified and its cells become irregular and star-shaped, these anastomose with one another by processes which gradually thicken In this way there arises a syncytium of protoplasmic shafts with slitlike, free interspaces. In many places the free interspaces disappear, leaving a compact syncytial mass. The nuclei, which are scattered in the syncytium, multiply energetically by mitosis. The cytoplasm contains many rod-shaped mitochondria which are frequently gathered in groups.

The development of myofibrils proceeds here as in striated muscle Homogeneous fine threads appear in the cytoplasm; they run for long distances quite independently of any cell territories. Then swellings appear at regular distances along these threads; they are the primordia of the A disks and are immediately followed by a second series of swellings between the first ones—the Z disk primordia Olivo has studied this in detail in tissue culture.

The myofibrils increase in number by splitting longitudinally. This causes the formation of bun-

Fig. 148 Large, centrally vacuolated Purkinje cells of human heart. The myofibrils are concentrated at the periphery of the cells. 950 ×. Drawn by Miss E. Bohlman.

cell membrane and which seem to have a partially continuous protoplasm and common fibrils, may beat with independent rhythms (Fig. 146). This observation offers a fairly strong argument in favor of the view that the cardiac muscle cells are independent cells. It shows, at least, that an apparent syncytium can break down into separate cells. Although the cardiac muscle cells may have a certain

Fig. 146. Two cells from a seven-day culture of the heart of a four-day chick embryo. The two cells were beating with different rhythms. Two smooth fibrils continue from one cell into the other. There is no distinct cell membrane separating the two cells. The dark granules are mitochondria. Stained with Janus green and fixed with iodine. 1450 ×. Courtesy of W. H. Lewis.

degree of morphological continuity, they are obviously discontinuous functionally—otherwise it is difficult to see how the two cells in Fig. 146 could beat with different rhythms.

Purkinje Fibers. Fibers of the Impulse-conducting System. Under the endocardium which lines the internal surface of the heart, particularly of the interventricular septum, there is a net of atypical muscle fibers. They are called "Purkinje fibers" after the man who discovered them.

It is now established that just as in the ordinary cardiac muscle, the Purkinje fibers form a continuous, sarcoplasmic network. It appears to be divided into separate sections because of the extremely irregular arrangement of the continuous bundles of striated fibrils; these pass mainly in the peripheral portions of the fibers. These are also provided with intercalated disks. Large amounts of sarcoplasm are accumulated about the nuclei. The sarcoplasm of the fibers of Purkinje often has a large amount of glycogen, particularly in children. In many places a gradual passage of these Purkinje fibers into ordinary cardiac fibers can be noticed.

The Purkinje fibers are laid down very early in the embryo and at once differ sharply from the ordinary elements of the cardiac muscle.

The coordinated contractions of separate parts of the cardiac muscle depend on the existence of a mechanism for conducting the stimuli for contraction. The fibers of Purkinje form part of this mechanism. Nerves may also participate in the conduction of the contractile impulse (see p. 250).

The sino-atrial and atrio-ventricular nodes have the same structure as the Purkinje fibers.

Connective Tissue and Blood Vessels of Cardiac Muscle. Loose connective tissue is found in the slitlike spaces of the cardiac muscle. The muscle fibers are everywhere surrounded by dense, basket-like networks of blood capillaries. These arise from the coronary arteries and are collected in the cardiac veins.

Histogenesis of Cardiac Muscular Tissue. The cardiac muscular tissue in the vertebrate embryo is formed from a portion of the splanchnopleure, which adjoins the exterior of the primordium of the heart—at this time a thin-walled,

cate that in contraction it decreases by ½ of 1 per cent of its volume. During contraction the longitudinal striation becomes more pronounced while the transverse striations become thinner and much closer to one another. At present most histologists believe that the most active and important rôle in the process of contraction is played by the myofibrils. That is, the physical and chemical changes which take place in the fibers during contraction and relaxation become manifest as changes in the cross striation of the myofibrils as seen in sections.

In the normal contraction of striated muscles in the warm-blooded animals, the contraction wave is so long and rapid that the entire muscle shortens and thickens at once. In order for all the fibers of a muscle to contract simultaneously they are provided with numerous nerve endings scattered along their length.

When a living muscle is teased under the microscope, its fibers are seen to contract for a long time. On fixation, the entire fiber may contract or agonal, short, local waves of contraction may become fixed in the characteristic spindle shape One can thus follow all the steps in the change from the resting, thin, cylindrical fibers into the condition of maximal contraction when they are thick and spheroidal.

In fixed preparations of local contraction waves, at the beginning of contraction, the fibrils begin to thicken, the disks become wider and thinner; Indeed, the I disk may become so thin that it disappears completely. When the shortening of the fibrils of the fiber reaches 50 per cent or more, the reversal of striation takes place. This consists in the appearance of thick, dark, transverse stripes which are located in place of the Z disk. According to some this is because the darkly stained substance of the A disk spreads in both directions from the middle disk M, penetrates the I disk and closely approaches the Z disk During these changes the double refraction of the A disk remains unchanged. The anisotropic substance maintains its outlines and its previous location and only flattens and widens, although the disk itself does not stain any more and loses its definite boundaries. During relaxation, that is, the return to the resting stage, these changes are repeated in the reverse order.

The main part of the process of contraction has as its morphological substratum the A and I disks The M and especially the Z disks apparently do not take an active part in contraction. They are transverse, elastic partitions attached to the sarcolemma; they are believed to support the entire fiber by interfering with the shifting of fibrils with relation to one another.

The *sarcoplasm must take part in the nutrition* of myofibrils, as it completely surrounds them. The sarcoplasm probably plays an important rôle in conducting excitation through the muscle fiber.

Mechanism of Muscular Contraction. The mechanism of the contraction of muscle is partially known. The evidence from studies with the *x*-rays and double refraction indicates that there is a definite molecular orientation, especially of stretched out protein molecules, in parallel chains. It is believed that during contraction, owing to chemical changes at the side groups of the proteins, these chains crumple and shorten in the longitudinal direction; the reverse change takes place in relaxation.

Chemical analyses of contracting muscle have shown that its carbohydrate breaks down into lactic acid; its combined phosphates also break down and one of the nucleotides becomes deaminized. Contradictory theories have been built upon various of these chemical changes, attributing the actual shortening to the formation of acidity in the one case and of alkalinity in the other at critical "contractile points." These contractile points would now be regarded as the side chains of the protein molecules mentioned above. (See review by Sacks.) On the basis of ultraviolet absorption spectra of striated muscle, Caspersson and Thorell claim that the main part of the adenine derivatives (adenylic and adenyl-pyrophosphoric acids) in resting muscle are localized in the I disks and that most of the myosin is in the A disks. They suggest that as a result of chemical processes taking place in the I disks, energy is transferred to the A disks which are the seat of the contractile elements.

Participation of Nerves in Muscular Contraction. All muscles are under more or less continuous nervous stimulation whose nature varies with the type of muscle and its nervous connections. Smooth and cardiac muscle are under autonomic nervous control and often contain autonomic nerve cells in their substance. The striated skeletal muscles, which are primarily concerned with control of movements and posture of the body and limbs, receive their motor impulses from the central nervous system by way of craniospinal nerves. The current belief that these muscle fibers receive an additional innervation from the autonomic system is not supported by the most careful recent work

When a skeletal muscle, preferably of a cold-blooded animal to avoid the effect of cooling, is removed from the body and its attached nerve is stimulated by a single electric shock, the muscle contracts quickly and relaxes almost imme-

other at various places; in this manner a network of cylindrical or prismatic muscle fibers is produced. Interstitial connective tissue penetrates the spaces between the splitting and separating portions of the syncytium.

The Purkinje fibers develop from the same primary syncytium as the ordinary cardiac muscle fibers. They soon become distinguishable from the remaining myocardiac mass as thick protoplasmic shafts swollen about the nuclei; they lie directly under the endocardium. A few myofibrils are irregularly distributed at their periphery.

Toward the end of the embryonic period, cardiac muscular tissue with its characteristic structure is well differentiated. But it must grow for a long time until the organism attains the completely mature state. How this takes place is not yet clear.

The increase in number of myofibrils and the thickening of separate fibers proceed by continued, longitudinal splitting of myofibrils At the same time the amount of axial sarcoplasm in the fibers diminishes correspondingly. It is also clear that the nuclei increase at first by mitosis and later perhaps by amitosis. The manner in which the fibers of the myocardium increase in number is as yet unknown, although it has been supposed that this also happens by longitudinal splitting of the existing fibers. Nothing is known of the details of the growth in length of the fibers and their fibrils; the participation of the intercalated disks in their formation is only a hypothesis.

In various pathologic conditions in the adult organism, an increase in volume of the cardiac muscle may take place. This probably depends in part on the increase in thickness and length of the existing fibers and in part on continued splitting of the muscular network, in the same manner as in the normal development of the heart.

Regenerative Capacity of Cardiac Muscle. The regenerative capacity of cardiac muscle tissue is insignificant, being even less than that of skeletal muscle. When marked degenerative changes occur around an injured area, healing takes place by the formation of scar tissue. A slight indication of regeneration is amitosis of the nuclei in cardiac muscle fibers and the development from them of special "sarcoblasts" or "myogenous cells." However, these never produce new fibers and always remain within the interstitial tissue. In certain pathologic processes in man (syphilis, diphtheria) the appearance of spindle-shaped myoblasts has been observed, which may also be regarded as an effort to regenerate. The peculiar large cells of the Aschoff nodules in the myocardium in rheumatic fever are no longer thought to arise from the muscle fibers. A yellowish pigment, possibly an abnormal metabolite, accumulates in smooth, cardiac and skeletal muscle of rats on a diet deficient in vitamin E.

Morphological Changes During Contraction of Muscle Fibers. The process of contraction can be watched directly if a bit of fresh smooth muscle, in a drop of physiologic saline, is stimulated by an electric current. Here each of the spindle-shaped cells shortens considerably and becomes thicker. In the spontaneous contraction of smooth muscle sarcoplasm flows to a central point which thickens while the nucleus and the mitochondria move passively. No fibrils can be seen in such living cells. It has been claimed that when smooth muscle contracts, the contracted portion is milky as long as the contraction lasts and then becomes translucent again with relaxation. When living smooth muscle is fixed, preparations are often obtained in which certain cells are fixed in contraction while adjacent cells are in relaxation. In fixed preparations, waves and nodes of contraction may be seen in the smooth muscle fibers; these may cover only a small portion of the cell and sometimes only a few fibrils (Fig. 149). The double refraction of the fibrils appears only in these contracted places while the internodal portions are isotropic.

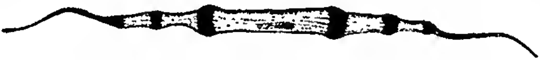


Fig. 149. A smooth muscle cell from the stomach of a sparrow showing six short contraction waves. Redrawn after Soli, from Maximow.

The ability of striated muscle fibers to contract and relax rapidly is generally believed to depend on the transverse striation of the fibrils, that is, on the distribution of the contractile substance in numerous separate small particles. Tissue culture experiments have shown, however, that cardiac muscle cells may lose their cross striations and still continue to contract rapidly.

In contracting, the striated muscle fiber, as well as the entire muscle, may shorten to as little as one tenth of its original length. Its thickness increases greatly and its volume probably remains unchanged; although some authors claim that it increases, the latest measurements indi-

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diately. This process is called a "twitch." When the nerve is stimulated by a rapid series of shocks, the muscle remains contracted and the condition is called "tetanus." The muscles are in this condition when executing any voluntary movements.

In the body, change in the position of any of its parts, such as occurs in executing a series of complex movements, depends on a constant series of nervous impulses mediated by the myelinated efferent nerves. These cause a beautifully coordinated series of contractions and relaxations of the antagonistic muscles involved.

In addition to this neuromuscular mechanism which controls the movements of the limbs, etc., and is based on typical reflex arcs (p. ???), there is another mechanism which controls the position of the limbs in space. This involves a series of unconscious, involuntary proprioceptive reflexes from the muscles themselves (see section on muscle spindles) from pressure organs, the labyrinths, eyes, etc. These are mediated through the central nervous system by myelinated nerve fibers and probably affect the same muscles as those involved in the voluntary movements. The impulses responsible for this "tone" are carried by the same nerves as carry the voluntary impulses. This is clearly shown in recent experiments in which a nerve bundle was dissected until only one fiber was left undivided. When stimulated, this nerve fiber caused the muscle to contract and when not stimulated, it still carried sufficient impulses to maintain the tone of the muscle. Thus, it appears highly probable that tone in skeletal muscle depends on the contraction of a few fibers. The normal stimulus for tone is probably in part the stretch which is put on the fibers as a result of the position the members of the body occupy after all movements. Those voluntary muscles which normally oppose the pull of gravity show tonus except during deep sleep and general anesthesia.

Cardiac muscle occupies a position intermediate between the extremes of skeletal and smooth muscle. It is striated in much the same way as skeletal muscle and its contraction is fairly quick. But it has, in addition, the ability to contract rhythmically and does not develop fatigue—its rest periods are slightly longer than its periods of contraction. It is not under voluntary control. Its rhythmical activity must arise originally as an intrinsic characteristic of the muscle, for in embryos the heart contracts for several days before any nerves have reached it. Moreover, in tissue cultures of embryonic heart muscle, individual muscle cells which have wandered into the plasma have been observed to beat with characteristic rhythms. Indeed, two apparently connected cells may have individual rhythms.

Microdissection studies by de Renyi and Hogue (1938) show that both myofibrils and sarcoplasm of living embryonic chick cardiac muscle cells in culture are contractile. Under normal conditions both contract synchronously, but when the sarcoplasm is paralyzed by the injection of certain reagents, the myofibrils continue to be active.

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The cells forming the nervous system are called nerve cells or *neurons* (Fig. 150). They possess a body made up of a nucleus and surrounding cytoplasm (*perikaryon*) which expands into a number of processes. These usually comprise several short dendrites and only one *axis cylinder*, or *axon*, which may have a great length (Fig. 151).

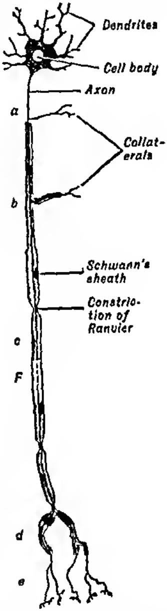

Fig. 150. Diagram of a peripheral motor neuron. *a*, area of the naked axon; *b*, area of the axon invested only with myelin; *c*, area covered with both myelin and Schwann's membrane; *F*, broken lines indicating great extent of the fiber; *d*, area in which the axis cylinder is covered only with Schwann's sheath and its nuclei; e, the area of the naked axis cylinder ending.

The size, shape and other peculiarities of the body, and the number and mode of branching of the processes vary in the extreme, producing countless varieties of nerve cells (Figs. 151, 158–160, 193, 534, 537, 539). It has been assumed that with the morphological diversity there goes some sort of functional specialization. The neurons are related anatomically and functionally by their expansions which are in contact with other nerve cells, or with epithelial, muscular, or glandular cells.

The point of contact of two nerve cells, called *synapse*, is more than a mere physical contact, being in fact a polarized organelle through which functional influence passes from one cell to another in one direction only (p. 209). Nearly every nerve cell possesses several or many synapses with other cells. If the nervous system were organized as a syncytium without inner boundaries, the impulses would spread in all parts without restraint. The evidence—morphological, physiological and pathological—indicates that the nervous system is built of countless cellular individuals that are structurally and functionally independent to a greater or lesser degree. The extreme multiplicity of forms and interrelationships of the nerve cells is a reflection of the complexity of functions mediated by this tissue.

THE MINUTE STRUCTURE OF THE NEURON

THE NERVE CELL

The nerve cell or neuron has a body containing a nucleus and threadlike processes or expansions. Often the mass of the cytoplasm in the processes is much greater than that in the cell body. The superficial zone of the cytoplasm appears to be of somewhat different character than the core of the cell body and evidently plays an important rôle in the transmission of the nervous excitations.

Chapter IX

THE NERVOUS TISSUE

The entire mass of nervous tissue in the body is called the nervous system. The essential function of this tissue is to receive stimuli from the environment, to transform these into nervous excitations and to transmit them to the nervous centers where they are reorganized to call forth appropriate responses. By these means the nervous system adjusts the activities of the individual to the events of the world in which it lives and so coordinates the functions of the various organs that they maintain the integrity of the body. The nervous system also includes the specific apparatus of all conscious experience. It is the dominant mechanism for the regulation of behavior and the maintenance of unity of the personality.

The nerve cells scattered in the various organs of the body and interconnected by nerve fibers, together with the nerves that link them with the brain and spinal cord, form the *peripheral nervous system*. The latter regulates the functions of the viscera and transmits to the peripheral organs regulating impulses from the brain and spinal cord. In the brain and spinal cord, the *central nervous system*, nervous impulses from all parts of the body come together and are integrated with other nervous impulses resulting from stimuli coming from outside the body. In this way, all parts of the organism are brought under a central authority which controls the activity of the organism as a biological entity.

The function of the nervous system is based on two fundamental properties of living substance. The first is the ability to react to various physical and chemical agents. The second is the ability to transmit the excitations thus elicited from one locality to another. The first property is called *irritability;* the second, *conductivity.*

The complex nervous system of the higher organisms evolved from these primitive properties of living substance in lowly forms. In the Metazoa certain cells developed the properties of irritability and conductivity to a high degree, forming a rudimentary nervous system. By further specialization some of the nerve cells evolved the capacity to react to special kinds of exogenous energy. These cells, with the corresponding accessory structures distributed througbout the body or near its surface, produced three sensory systems: the *exteroceptive system*, concerned with receiving impulses from the surface of the body; the *interoceptive system*, responsive to impulses from the internal organs; and the *proprioceptive,* which receives excitations from the muscles, tendons and joints. Other nerve cells became connected with the peripheral *effector organs,* as the muscles and glands, forming the *neuromotor* and *secretory systems*. Still other nerve cells, mostly collected in a large, central mass, assumed the role of *correlators* or *integrators.* These receive, select, combine, distribute, inhibit or otherwise modify the excitations arriving from the receptive surfaces or from the inner organs, and finally influence properly the peripheral effectors.

The functional significance of the neurofibrils is not yet clear. In the opinion of some the neurofibrils are the substrate for the transmission of the nervous excitations; others consider them to be merely supporting structures. According to most investigators, the neurofibrils are confined to the territories of their respective neurons (see Bartelmez and Hoerr; Bodian; and Fig. 191).

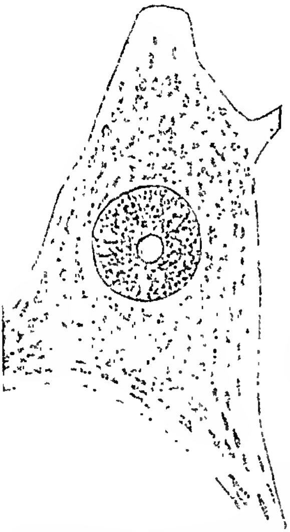

Fig 152. Motor cell from the gray substance of the ventral horn of the spinal cord of a cat showing granular chromophil substance. Axon. 670 ×. (A.A.M.)

The Neuroplasm. The *neuroplasm* is the undifferentiated part of the cytoplasm of the nerve cells wherein the neurofibrils are embedded. Neuroplasm in the processes is called interfibrillar substance; in the axis cylinder it is called axoplasm. In fresh condition the axoplasm appears homogeneous; in the dark field it contains scattered bright granules.

Chromophil Substance. The *chromophil substance*, or *Nissl's bodies* or *granules* (Figs. 152, 155, 191, 201) are conspicuous structures of the nerve cells and show important changes in some pathological conditions. Nissl's granules, invisible in living or fresh material, are best demonstrated by one of the selective methods of staining devised by Nissl, as toluidin or thionine blue or other basic aniline dyes. Thus treated, the granules appear intensively stained, much like the chromatin of the nucleus; the intervening channels are filled with the neurofibrils which in Nissl's technic remain practically unstained (compare Figs. 152 and 153).

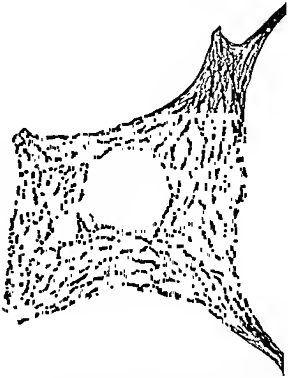

Fig. 153. Motor cell of the ventral gray column of the spinal cord of a rabbit; a net of neurofibrils is seen in the perikaryon and continuing into the processes; the nucleus appears as a pale disk. Bielschowsky-method. 500 ×. (A.A.M.)

The physiological significance of the chromophil substance is still undeter-

Nucleus. The globular nucleus is relatively large, though it varies with the volume of the cell body and apparently fluctuates with phases of life or activity of the cell. The usual position of the nucleus is in the center of the body. A nuclear membrane is always present. The linin framework is prominent, its meshes being filled with a large quantity of nuclear sap. Characteristically, there is one relatively large oxyphil nucleolus; rarely are there several nucleoli. The basichromatin is scanty, and the nuclei of the nerve cells appear as pale vesicles.

Body or Perikaryon. The cytoplasm that constitutes the body of a nerve cell is differentiated into several structures. These are: (1) neurofibrils, (2) interfibrillar substance, (3) chromophil substance or Nissl's bodies, (4) mitochondria, (5) internal reticular apparatus of Golgi, (6) centrosome, and (7) various inclusions. Special methods must be used to demonstrate most of these structures, although neurofibrils and mitochondria have been seen in fresh cells.

Neurofibrils. The *neurofibrils* are best developed in large neurons, but their presence has been demonstrated in almost every variety of nerve cell. They appear as homogeneous threads, embedded in a more liquid protoplasm (Fig. 153). The neurofibrils are distributed as a complicated network throughout the cell body, and spread into all processes, where they can be followed into the finest terminal ramifications (Figs. 174, 175, 192).

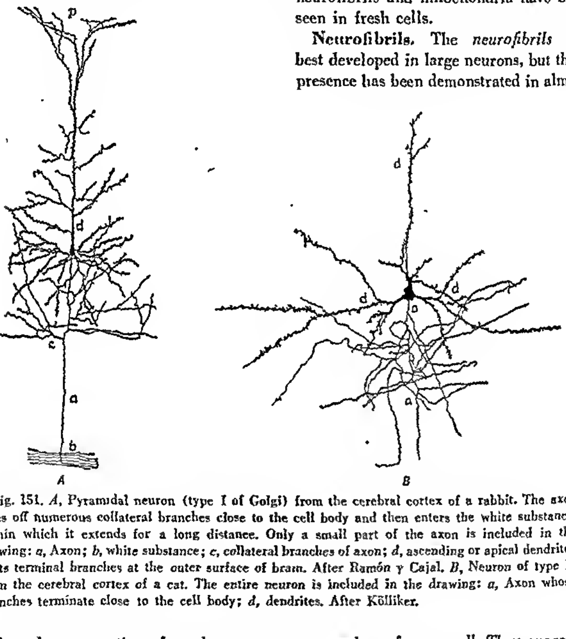

Fig. 151. *A*, Pyramidal neuron (type I of Golgi) from the cerebral cortex of a rabbit. The axon gives off numerous collateral branches close to the cell body and then enters the white substance, within which it extends for a long distance. Only a small part of the axon is included in the drawing: *a*, Axon; *b*, white substance; *c*, collateral branches of axon; *d*, ascending or apical dendrite; *p*, its terminal branches at the outer surface of brain. After Ramón y Cajal. *B*, Neuron of type II from the cerebral cortex of a cat. The entire neuron is included in the drawing: *a*, Axon whose branches terminate close to the cell body; *d*, dendrites. After Kölliker.

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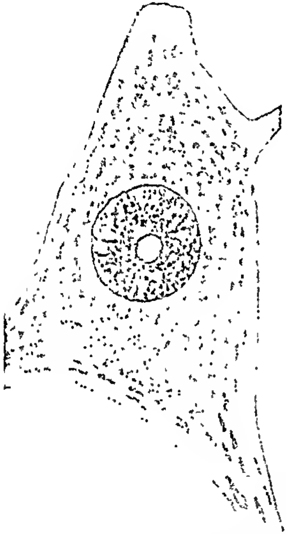

Fig 152. Motor cell from the gray substance of the ventral horn of the spinal cord of a cat showing granular chromophil substance. Axon. 670 ×. (A.A.M.)

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Chromophil Substance. The *chromophil substance,* or *Nissl's bodies or granules* (Figs. 152, 155, 191, 201) are conspicuous structures of the nerve cells and show important changes in some pathological conditions. Nissl's granules, invisible in living or fresh material, are

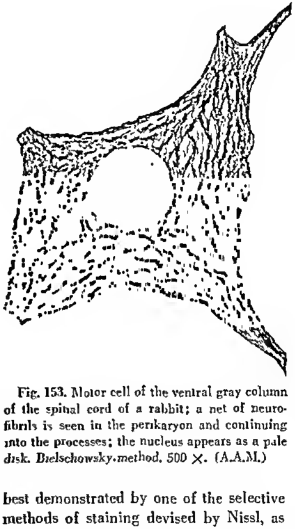

Fig. 153. Motor cell of the ventral gray column of the spinal cord of a rabbit; a net of neurofibrils is seen in the perikaryon and continuing into the processes; the nucleus appears as a pale disk. Bielschowsky method. 500 ×. (A.A.M.)

best demonstrated by one of the selective methods of staining devised by Nissl, as toluidin or thionine blue or other basic aniline dyes. Thus treated, the granules appear intensively stained, much like the chromatin of the nucleus; the intervening channels are filled with the neurofibrils which in Nissl's technic remain practically unstained (compare Figs. 152 and 153).

The physiological significance of the chromophil substance is still undeter-

mined. It is absent from certain neurons and from the axis cylinders. Possibly it represents reserve material easily utilizable during the activity of the nerve cell —a view supported by the marked changes observed in the chromophil granules under varying physiological and pathological conditions.

The chromophil granules are distributed in the entire cell body except in its most peripheral layer and the zone immediately adjacent to the nucleus. They are also present in the dendrites but absent from the axon and its origin from *the cell body, the axon hillock. The form, size,* and distribution of the chromophil granules vary in the extreme, appearing in as many patterns as there are varieties of nerve cells (Nissl). As a rule they are coarser and more abundant in large cells, especially motor ones, and quite scarce and fine in small cells of the granular type. Under different physiological conditions, as rest and fatigue, the granules change their aspect. In pathological processes they may be dissolved and disappear. Their disappearance may be the consequence either of direct injury to the cell body or to the axis cylinder anywhere along its course, and is called *chromatolysis or tigrolysis* (Fig. 201); the process that brings it about, if caused by injury to the axon, is called Nissl's reaction or retrograde degeneration, or primary irritation of the nerve cell (p. 220).

The study of Bensley and Gersh with the freezing-drying method is in favor of the view that *the Nissl substance is not distributed homogeneously in the living cell* (Fig. 155). With the aid of ultraviolet microscopy and the use of ribonuclease, Gersh and Bodian have shown that ribose nucleoprotein is one of the main constituents of the Nissl substance. Hydén, using similar technics, *believes in a nucleolar origin of this* material.

Golgi Apparatus. The *intracellular reticular apparatus of Golgi* (Fig. 156) is present in all nerve cells and appears as a network of black, *irregular, wavy threads and bands, coarser* than the neurofibrillar network. The form of this organoid varies considerably in different types of neurons.

It is necessary to point out the difference between this intracellular reticular structure and another network, the *pericellular net of Cajal and Golgi.* The latter surrounds the body of the nerve cell from outside; it is essentially of neuroglial origin and is, therefore, not part of the neuron.

Mitochondria. The *mitochondria* are minute rodlike or filamentous structures scattered everywhere between the Nissl's granules and neurofibrils (Figs. 157, 163). They can be demonstrated in many fresh nerve cells by supravital staining. Their number varies from a few to many.

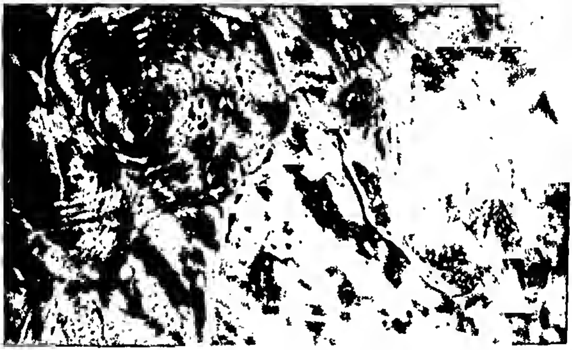

Fig. 154 Two bipolar cells of a ganglion from an eight-day chick embryo cultivated in vitro for seven days. Unretouched photograph of the living cells showing neurofibrils. 1650 ×. After Weiss and Wang, 1937. (Courtesy of The Wistar Press.)

Centrosome. The *centrosome* is a spherical structure characteristic of the immature, multiplying nerve cells during the early stages of embryonic development. In fully developed, static, adult neurons, especially of the vertebrates, a typical centrosome is seen but rarely. Most of the structures so designated have another significance

Inclusions. Besides the structures mentioned, there are inclusions in the nerve cells which are less widespread and less constant. Bright *vacuoles* have been described in the fresh nerve cells of lower animals. *Pigment granules* are frequently encountered. The coarse, dark-brown or almost black granules are undoubtedly melanin and are found in certain cells only, thus in the substantia nigra of the midbrain, in the locus coeruleus in the floor of the fourth ventricle, in

Fig. 155 High power photomicrograph of anterior horn nerve cell of the spinal cord of the rabbit, fixed by the freezing-drying method, untreated, undenatured, and stained with toluidin blue. After Bensley and Gersh.

the dorsal vagus nucleus, and in the spinal and sympathetic ganglia Its physiological significance is unknown. More frequent, especially in man, are fine lipochrome granules of yellowish color. They are probably a product of normal activity which remains within the protoplasm in a useless although noninjurious capacity. In favor of this view is the gradual increase in the amount of the pigment with advancing age. *Fatty substances* are encountered in the form of inclusions in the protoplasm of the nerve cells either as reserve material or as a product of normal or pathological metabolism. *Glycogen* is found in the ependyma, chorioid plexus and nerve cells of the embryonic, but not in a demonstrable quantity in the adult nervous tissue. *Iron-containing granules* are found in the nerve cells of the substantia nigra, the globus pallidus, and elsewhere.

Processes or Expansions. The processes or expansions of the nerve cells are their most remarkable characteristics. In almost every one of the many varieties of neurons there are two kinds of processes: the dendrites and the axis cylinder.

The *dendrites* or the *protoplasmic processes* (Figs. 150) are direct expansions of the body. Nissl's chromophil granules and mitochrondria are found in the thicker portions of the dendrites (Figs. 152, 155, 203). A neuron usually has several main

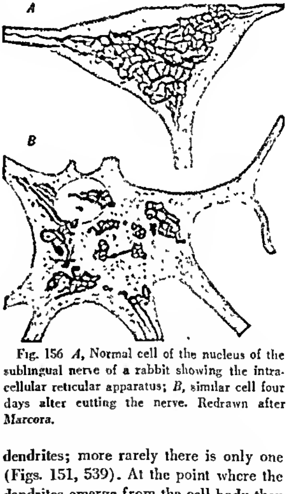

Fig. 156 *A*, Normal cell of the nucleus of the sublingual nerve of a rabbit showing the intracellular reticular apparatus; *B*, similar cell four days after cutting the nerve. Redrawn after Marcora.

dendrites; more rarely there is only one (Figs. 151, 539). At the point where the dendrites emerge from the cell body they are thick, rapidly becoming slender toward their ends. Each dendrite usually divides into primary, secondary, tertiary and more branchlets. These are of the most varying shapes and sizes, distributed in the most diverse ways; but they are typical for each variety of neuron. As seen in Golgi preparations the surface of many dendrites is covered with a great number

of minute, thorny "spines," also called "gemmules," giving the dendrites the appearance of a test-tube brush. These "spines" and other similar terminal twigs often serve as synaptic organs. In the majority of the neurons the dendrites are short and are confined to the immediate vicinity of the cell body. The number, length and arrangement of the terminal twigs of the dendrites vary in the extreme and are not directly dependent upon the size of the perikaryon. The pattern and the size of the dendrites are characteristic for each variety of neuron.

The dendrites, through their synapses with the axon endings of functionally related neurons, receive nervous impulses

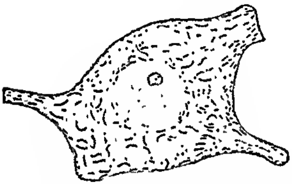

Fig. 157. Nerve cell body of a rabbit showing mitochondria Redrawn after Schirokogorow.

from other neurons. The dendrites are, with the perikaryon, the chief receptive organelles of the neuron.

The *axis cylinder or axon* differs considerably from the dendrites. While there are usually several dendrites, there is only one axis cylinder to each neuron (*a*, in Fig. 150). This process often arises from a small conical elevation on the cell body, devoid of Nissl's granules, called axon-hillock (Fig. 152). The axon does not contain Nissl's granules and usually is thinner and much longer than the dendrites of the same neuron. The axon never has "spines" and is, therefore, of smooth appearance. It becomes gradually, almost imperceptibly, more slender toward its end.

Along its considerable course the axis cylinder may or may not emit collateral branchlets (Fig. 150). The chief arborization, however, is at the end of the main branch and is called *axon ending* (also *telodendron*) (*e* in Fig. 150; Fig. 539). It is composed of primary, secondary, and other branches and buds varying greatly in number, shape and distribution. Often its branches are assembled into baskets that surround the body of the related neuron, or they twist around the dendrites of the latter. In simpler cases one or two twigs of an axon ending just touch the surface of a dendrite or the body of another related neuron (Figs. 193, 539).

The axon normally receives nervous excitations from its own cell body, and thus from its dendrites, or directly from the dendrites where these are continuous with the axis cylinder of its own neuron (as in the second s ganglion from the left, in Fig. 539). It transmits the excitation through its ending to other neurons or to effector cells, as muscle fibers or glandular cells. There are as many modes or types of axon endings as there are varieties of neurons (Figs. 193, 539). Moreover, the same axis cylinder may terminate in several different ways and be synaptically connected with several different neurons (Fig. 539).

Some exceptions to these features characterizing dendrites and axons are encountered, as in the peripheral sensory neurons of the spinal ganglions (Figs. 158, 160). In these the afferent fiber in the adult has all the earmarks of an axis cylinder although genetically and functionally it behaves in the same way as do the dendrites in most other neurons.

Forms and Varieties of Neurons. Depending on the number, length, thickness, and mode of branching of the processes, and also on the shape, size, and position of the cell body, and on the synaptic relationships, an infinite number of types of neurons can be distinguished in the nervous system. In general, the neurons may possess axis cylinders of considerable length that

leave the place of their origin in the gray substance and traverse the so-called "white or fibrous mass," or become peripheral nerve fibers, and terminate at some distance in another locality. Such are termed *Golgi type I* neuron with the

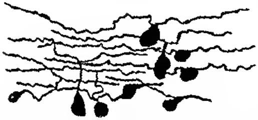

Fig. 158. A collection of cells from the ganglion of the trigeminus of the embryonic guinea pig, to illustrate various stages in the transformation of bipolar neuroblasts into unipolar ganglion cells. After van Gehuchten.

long axon (Figs. 151, *A*; 539, *m*, *n*, *o*, *p*, *s*). To this type belong all of the peripheral nerves and neurons whose axis cylinders form long fiber tracts of the brain and spinal cord. In other neurons the axis cylinder is relatively short and

The shape of the cell bodies is variable; it may be spherical, oval, piriform, spindle-shaped or polyhedral. The shape is determined by the mechanical pressure of surrounding structures, by the number, size, and place of origin of the processes, by the internal organization of the cell, but above all by the requirements of function. The absolute size of the neurons likewise varies between extreme limits, from midgets to those of giant size.

The *unipolar neurons* are the nerve cells that have a single process, the axon. They do not possess dendritic processes. In the central nervous system such neurons are rare, except in early embryonic stage (Figs. 199, 200). In the *bipolar neurons* each cell has one main dendrite and one axis cylinder projecting from opposite ends of the fusiform body. Typical bipolars are found in the retina (Figs. 537, 539), in the vestibular and cochlear ganglions, and in the olfactory nasal epithelium. In vertebrate embryos all neurons of the craniospinal ganglia are at first bipolar (Figs. 154, 198); during further development they undergo changes (Fig. 158), resulting in

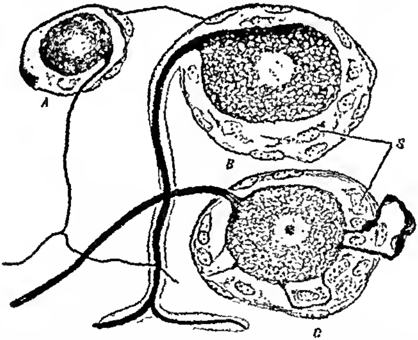

Fig. 159 Three cells from the nodose ganglion of the vagus nerve of man, A and B, with two T-shaped dividing processes: cp, Capsule; S, satellites; cell C has looped processes. Redrawn after Ramón y Cajal.

does not leave the confines of the gray substance where its body lies. These represent *Golgi type II* neuron with the short axon (Figs. 151, *B*; 539, *c*, *d*, *e*, *f*, *h*, *l*, *i*). Such neurons are especially numerous in the cerebral and cerebellar cortex and in the retina.

the peculiar forms described below. The single process shown by most of these cells does not represent a simple axon; because of this these elements may be called pseudo-unipolar (Figs. 159, 160).

In the *multipolar neurons*, representing by

far the great majority of neurons, the shape is *determined by the number and arrangement of* their dendrites (Figs. 150, 151). The *star-shaped neurons* are the motor cells of the ventral gray columns or anterior horns of the spinal cord (Figs. 152, 156). The *pyramidal neurons* (Figs. 151, *A*; 191, 201) are one of the characteristic elements of the cerebral cortex. In these a thick, trunklike dendrite projects from the upper end dendrites covered with a multitude of tiny "spines." The large dendritic tree-top is confined to a narrow zone; it resembles a richly arborized fan oriented across the longitudinal axis of one of the cerebellar convolutions and vertical to its surface. The axis cylinder enters the white subcortical mass.

Many more varieties are found both in the cerebral and cerebellar cortex and elsewhere,

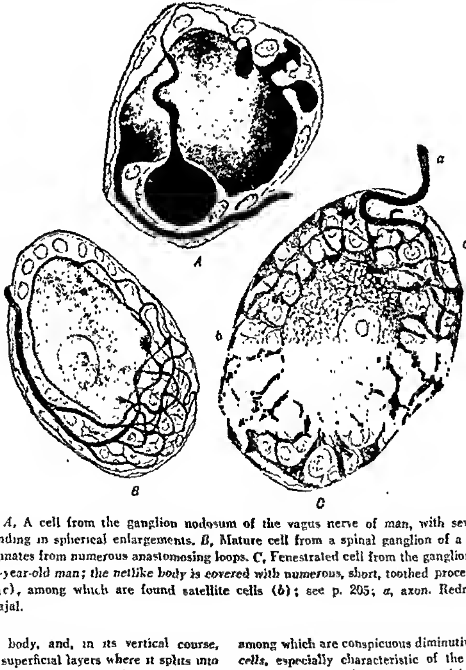

Fig. 160. *A*, A cell from the ganglion nodosum of the vagus nerve of man, with several short processes ending in spherical enlargements. *B*, Mature cell from a spinal ganglion of a sheep; its process originates from numerous anastomosing loops. C, Fenestrated cell from the ganglion nodosum of a seventy-year-old man; the netlike body is covered with numerous, short, toothed processes of the cytoplasm (*c*), among which are found satellite cells (*b*); see p. 205; *a*, axon. Redrawn after Ramón y Cajal.

of the cell body, and, in its vertical course, reaches the superficial layers where it splits into a tassel, the so-called "apical dendrite." Other dendrites arise from the lower end of the body—the basal dendrites. The axis cylinder emerges from the base and passes into the subcortical white mass.

Of remarkable shape are *Purkinje's cells in the* cerebellar *cortex*. In these, from the upper end of the body arise two thick, rapidly dividing among which are conspicuous diminutive *granule cells*, especially characteristic of the receptive sensory areas, as the striate area (visual), the region around the central sulcus (part of the sensory-motor cortex) and of the transverse temporal convolutions in the Sylvian fossa (auditory). In these the few short dendrites radiate in all *directions*, while the axis *cylinder and its* branches are also confined to the immediate neighborhood of the cell. This variety exemplifies

neuron type II of Golgi with the short axon. To the same type belong also many other neurons, as the asteriform and basket cells in the cerebellar cortex and the horizontal cells in the retina (Fig. 539, *c*).

Of special interest are the ganglion cells of [illegible] of the cranial and spinal nerves [illegible]

a single process arises which divides like the letter T into a peripheral or dendritic branch that becomes the axon of a peripheral sensory nerve fiber, and into a central or axonic branch which is the axon of a sensory posterior root fiber that terminates in the central nervous system. Although unipolar, these neurons are physiologically bipolar. The initial single expansion and both central and peripheral branches are enveloped in myelin and Schwann's sheaths. The body of each cell is enveloped by two cellular capsules. The inner is made up of small, flat, epithelium like satellites or Schwann's cells continuous with similar cells enveloping the peripheral processes. The outer capsule is formed by a special modification of the interstitial connective tissue, with a thin structureless membrane as an inner layer. A dense capillary network is present in the capsule of each cell. The capsule is made up of collagenous fibers and fibroblasts arranged concentrically. It extends along the cellular process, becoming continuous with the endoneurium of the nerve fiber.

The few examples described above give but an incomplete picture of the wealth of the varieties of neurons. *Many more have been discovered by* numerous investigators, especially by Ramón y Cajal and his pupils, but probably many more remain unknown. It is apparent from the above that each ganglion or cortical area is composed of a multitude of varieties of neurons differing from place to place, and occurring side by side.

THE NERVE FIBER

The nerve fiber, in the conventional sense (Fig. 161), is the axis cylinder or axon of a nerve cell with its enveloping neurolemmal and myelin sheaths (where the latter is present). In the fresh condition the nerve fiber appears as a homogeneous, shiny, and slightly yellowish tube with thick walls. Various histological technics reveal in its center a continuous smooth gray stripe, the *axis cylinder*, wrapped in one or two sheaths (Fig. 161). In cross sections the unstained myelinated nerve fibers appear as small or large circles with sharp outlines formed by the highly refractive neurolemmal sheaths, and with darker spots in their centers which are the *axis cylinders* (Figs. 168, 171). The myelin sheaths in untreated preparations remain invisible. The appearance of the various constituents of the nerve fiber differs according to the technic applied: the vital methylene-blue and the various silver methods stain the axis cylinder blue, brown or black; Weigert's *and* similar *methods stain* the myelin sheath alone.

The Axis Cylinder. The axis cylinder or axon is a thin thread of fairly uniform thickness and smooth appearance. In the periphery, at fairly regular intervals, at the constrictions of Ranvier (p. 192), it is thinner than between the constrictions (Fig. 161). The *axon* is a direct continuation of the protoplasm of the cell body. It does not possess a special membrane of its own, nor is the superficial zone of its substance denser than its core. At Ranvier's constrictions its substance is more viscous or solid than in the interstrictural portions.

The fresh axis cylinder appears homogeneous. However, when treated with selective methods (vital methylene blue, *methods of Cajal* or *Bielschowsky*, observation of living tissue cultures), the presence of *neurofibrils* and of the undifferentiated axoplasm is revealed. Each neurofibril is directly continuous with the neurofibrillar network of the cell body. There are no Nissl granules in the axis cylinders. Some have reported the presence of mitochondria in the axon near its origin on the cell body.

The axis cylinder conducts nervous impulses *from the body of* a neuron, and thus from its receptive dendrites, to some effector organ, a muscle or a gland. In the process of conduction the enveloping sheaths act as insulators. The axis cylinder carries the nervous influences to dis-

tant regions of the body—as from the cells of the cerebral cortex to the motor cells of the anterior horns of the spinal cord, and, as the peripheral neuron, from these to the various muscles, and other tissues.

Schwann's Sheath or Neurolemma. This is a delicate, transparent, nonelastic, tubelike membrane which, in the peripheral nerve fibers, envelops the axis cylinder (Figs. 150, 161, 162). In those nerve fibers that also possess a myelin sheath the neurolemma is the outermost. It is composed of *Schwann's cells* that, like the links of a chain, follow one another along every peripheral nerve fiber from its beginning, at the spinal root or in some ganglion, almost to its peripheral termination. Each Schwann's cell, with its flat and oval nucleus surrounded by protoplasm containing a Golgi net and mitochondria (Fig. 161, *p* and *s* in Fig. 162), envelops a segment of axis cylinder. In myelinated nerve fibers the consecutive segments are separated from one another by a *constriction of Ranvier*, where the adjoining segments merge (Fig. 161). The neurolemma does not belong to the connective tissue but is a membrane similar to sarcolemma. It occasionally contains granular inclusions of complex lipoids which increase greatly with advancing age and even more so in pathological conditions.

Like the neurons, Schwann's cells are of ectodermal origin. They can be considered as the "peripheral neuroglia" that have left the central nervous system and have become adapted to the special conditions of the peripheral nervous system (Harrison). As the peripheral axons grow, embryonic cells of Schwann follow them, *enveloping one segment after another, and freely migrate* from branch to branch or *from axon to axon*, until they form the complete neurolemmal sheaths (Speidel).

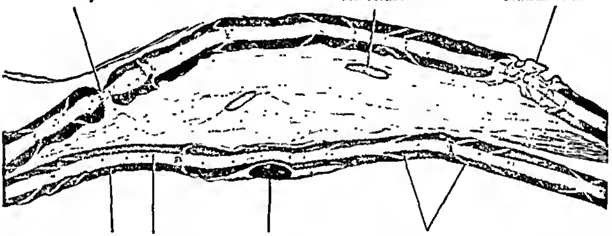

Fig. 161. Two myelinated fibers of the sciatic nerve of a frog; treated with osmic acid and picrocarmine and teased. 330 ×. (A.A.M.)

Schwann's cells are indispensable for the life and function of the axons of the peripheral nerve fibers. They probably are important in the metabolism of the axon. In axons that do not possess myelin sheaths they possibly also serve as insulators, preventing the diffusion of the nerve current passing through the axon. In the process of *regeneration* the newly built axon always grows out of the central stump which remains *continuous* with the cell body of the neuron, and spreads along the bridges formed by Schwann's cells (p. 222). In tissue cultures, Schwann's cells may transform into macrophages.

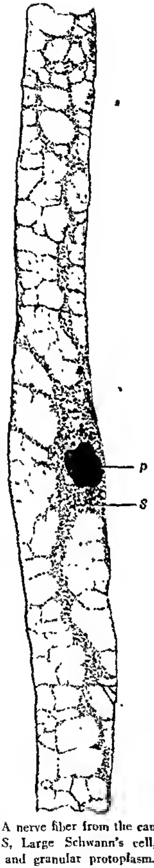

Fig 162. A nerve fiber from the cauda equina of a cat: *S*, Large Schwann's cell, with its nucleus, *p*, and granular protoplasm. Redrawn after Nemiloff.

The Myelin Sheath. Nerve fibers which have a myelin sheath are called myelinaled or medullated nerve fibers (Fig. 161). The myelin is a complex, little known mixture of various lipoids, the most important being cholesterol, besides

Fig. 163. Myelinated nerve fiber from the central nervous system of a guinea pig In the center is the axis cylinder. In the protoplasmic network, which penetrates the myelin substance and forms a funnel shaped cleft, are granular mitochondria. Redrawn after Nageotte.

certain cerebrosides, phospholipins and fatty acids. Most of the myelin is soluble in lipoid solvents. In fresh unstained nerve fibers myelin forms a glistening en-

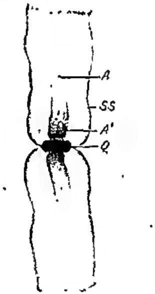

Fig. 161. Silvered, myelinated nerve fiber from the sciatic nerve of a frog showing a cross of Ranvier: *Q*, Cross plate (cementing ring), *A'*, spiny bracelet of Nageotte; *A*, axis cylinder; *SS*, neurolemma. 740 ×. After Schaffer.

velope around the axis cylinders. It is this property of myelin that is responsible for the white color of the fiber masses of the brain, called white substance, and of parts of the spinal cord and numerous periph-

eral nerves. The myelin sheath is non-elastic.

Schmitt, Bear and Palmer, on the basis of x-ray diffraction patterns, describe the myelin sheath as "being composed of concentrically wrapped layers of mixed lipides alternating with thin, possibly unimolecular, layers of neurokeratinogenic protein material. Within the layers the lipide molecules are oriented with paraffin chains extending radially and with polar groups in the aqueous interfaces, loosely bonded to those of the protein. . . . The specific structure of the sheath is relatively insensitive to the action of temperature, electrolytes, and detergents."

In contrast to the continuous neurolemma, the myelin sheath of a nerve fiber is completely interrupted at fairly regular intervals by circular *constrictions of Ranvier* (also called "nodes"), and is thus divided into cylindrical segments These are shorter in the terminal portion of the fiber. The length varies in different nerve fibers and in different animals from 50 to 1000 microns. The thicker the fiber, the longer are the segments. Each segment consists of one neurolemmal cell of Schwann. The protoplasm of the cell is seen with some stains as a complex net of thick and thin trabecles pervading the entire myelin sheath as far as the axis cylinder (Fig. 163). Whether these trabecles merge into a distinct envelope, the axolemma of Mauthner, separating the axon from the myelin sheath, is not certain. If the peripheral nerve fiber gives off collateral branches this always takes place at a Ranvier's constriction (Fig. 170, *C*).

In fixed preparations of the peripheral nerves the myelin of each segment is interrupted by oblique partitions, the *incisions* or *clefts of Schmidt-Lantermann,* several to each Schwann's segment (Fig. 161). The clefts have been seen in teased fresh nerves of rats. Many nerve fibers in the brain and spinal cord, especially those that form the white subcortical substance, have myelin sheaths but lack the neurolemma, for which is substituted *neuroglia,* particularly the oligodendroglia (Figs. 165, 188, *D*). Both Ranvier's constrictions and Schmidt-Lantermann's clefts seem to be absent in the brain and spinal cord.

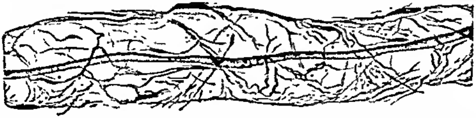

Fig. 165. A myelinated nerve fiber from the ventral column of the white matter of the spinal cord of a cat; the axis cylinder is surrounded by a myelin sheath enveloped by neuroglia fibers. Redrawn after Paladino.

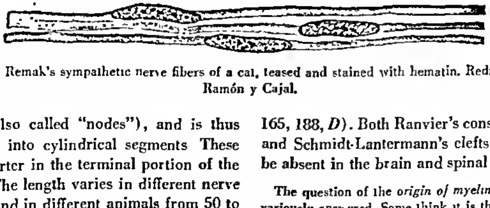

Fig. 166. Remak's sympathetic nerve fibers of a cat, teased and stained with hematin. Redrawn after Ramón y Cajal.

The question of the *origin of myelin* has been variously answered Some think it is the product of Schwann's neurolemmal cells (in the peripheral nervous system), or of the neuroglial cells (in the brain and spinal cord). Others consider that the myelin is the product of the axis cylinder and is, therefore, a part of the *neuron itself. However, certain observations,* as the fact that the bodies of cells of the spinal

ganglion of the acoustic nerve are enveloped by myelin husks whose structure resembles neuroglial husks elsewhere, are difficult to reconcile with the latter opinion. In any case, there is little doubt that the neurolemma and the neuroglia are indispensable for the formation of myelin (p. 218). Likewise, in pathological processes, whenever a neuron and its axis cylinder are affected, both the neurolemmal and the myelin sheaths undergo reactive changes; in the central nervous system the neuroglial cells react in addition to the myelin sheaths (p. 222).

The myelinated nerve fibers are characteristic of the vertebrate nervous system. In lower animals they are rarely present. During ontogenesis the myelin appears relatively late and the *process of myelinization* ends only some time after birth. Different fiber systems or tracts of the brain and spinal cord become myelinated at different times (Flechsig).

Numerous, mostly thin, axis cylinders in the peripheral nervous system, especially in the sympathetic, have only a neurolemmal sheath, the myelin being absent. These are gray in color and difficult to demonstrate with ordinary technic. They can be seen more easily when stained with the vital methylene-blue method or with silver. These unmyelinated nerve fibers are known as *Remak's fibers* (Figs. 166, 167). However, studies with polarized light indicate that many apparently naked fibers may possess traces of myelin.

The probable function of the myelin sheath is to improve the insulation of the axis cylinder against the loss of nervous current traversing it during its activity, as compared with the naked axon wrapped only in the neurolemmal sheath. Precisely how the insulation is achieved, and what chemical and physical interactions take place between the axon and the various sheaths during the active nervous process and in the exchange of nutritive materials are unknown.

Physiological Properties of the Nerve Fiber. The nerve fiber is essentially a highly irritable conductor. Along it the dynamic nervous excitation propagates in waves at a relatively high speed (up to 150 meters per second), faster in myelinated than in naked axons. During the conduction of excitation the activity of one portion of the axon serves as a stimulus activating the next portion, and so on. As the nerve fiber becomes active it changes its electric potential, the outside of each active portion becoming *negative* relative to resting portions. Action currents then flow between active and resting regions. When artificially stimulated the nerve fiber increases its metabolism (Gerard, 1932).

The following features are characteristic of the activity of the nerve fiber or axis cylinder: (1) Like the living substance in general, it possesses *irritability*, or the ability to respond to various stimulating agents, and *conductivity*, or the ability to transmit impulses from point to point. (2) To act as a transmitter the nerve fiber must be anatomically continuous and physiologically in an appropriate condition. (3) After the passing of the impulse the fiber remains for a short time unexcitable ("refractory period"). (4) Although under normal conditions the impulse always travels in one direction, that is, from the perikaryon or dendrites toward the axon ending, when artificially stimulated, an impulse propagates from the stimulated point in both directions along the axon, toward the perikaryon ("antidromic conduction") and away from it, toward the axon ending. (5) The impulse normally remains confined to the stimulated axon, spreading only along it and its branches (insulation of the impulse); it can reach other neurons through special points of contact, the synapses. (6) The impulse traveling along an axon can be weakened temporarily or blocked by the local action of heat, cold, pressure, electric current and by many drugs (anesthetics, narcotics). (7) Any stimulation intense enough to cause the axon to respond calls forth the maximum discharge of which the axon is capable ("all or

nothing law"). The varying intensity of the stimulus is expressed by the frequency of single like discharges.

The properties of simple nervous conductors, as listed above, differ profoundly in several respects from those of the synaptic gray nervous substance of the brain, the spinal cord and the ganglia, as discussed on p. 214.

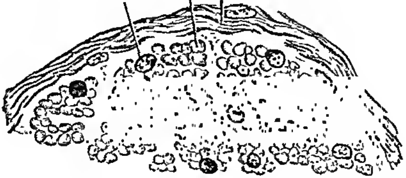

Fig. 167. A cross section of a sympathetic nerve of an ox. Redrawn after Ramón y Cajal.

forming peripheral *nerve trunks* and their branches (Figs. 167–171). Where most of the nerve fibers are myelinated, they are characteristically white and glistening in appearance, as in the craniospinal nerves.

The *white matter* of the brain, the various bundles of the brain stem, the core of the cerebellum and the white columns of the spinal cord, consist mainly of myelinated

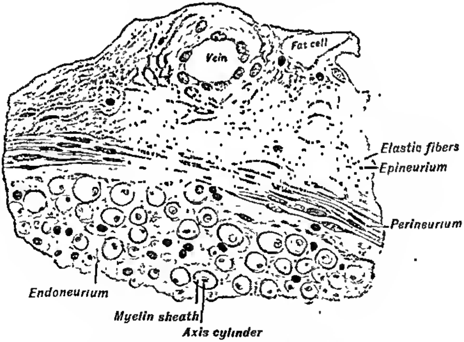

Fig. 168. Portion of a cross section through a branch of the median nerve of man. 380 ×. After Schaffer.

Nerve Fibers as Constituents of Peripheral Nerves, Brain and Spinal Cord. In their peripheral course outside the central nervous system, both myelinated and unmyelinated nerve fibers are bound into bundles by connective tissue, nerve fibers; here there are few, if any, nerve cells. The *gray matter*, found in both the cerebral and cerebellar cortex, in the basal ganglia of the brain, in the numerous nuclei of the brain stem, in the gray columns of the spinal cord, in the

peripheral ganglia and in the retina of the eyes, is chiefly composed of the bodies of numerous nerve cells, their dendrites, and the initial and terminal unmyelinated portions of axis cylinders. The nerve fibers of some sympathetic nerves are mostly Remak's unmyelinated gray fibers. Both white and gray matter contain neuroglia and blood vessels, although these elements are more abundant in the gray matter. In certain regions of the central nervous system the constituents of both white and gray substance are mixed in various degrees, as along the sides of the thalamus, in the subthalamus and hypothalamus, in the tegmentum of the brain stem and at the junction of the anterior and posterior gray horns of the spinal cord. Such regions are called *reticulate substance* or *reticular formation.*

The *peripheral nerves* are composed of fascicles of nerve fibers of varying thickness (1, 2 and up to 30 microns) and of varying degrees of myelinization, with or without unmyelinated fibers, held together by connective tissue. The outer layer of the latter, the *epineurium* (Figs. 168, 169), is made up of connective tissue cells and of collagenous fibers, mainly arranged longitudinally. Fat cells may also be found here. Each of the smaller fascicles of a nerve is in turn enclosed in a membrane of dense, concentric layers of connective tissue called *perineurium* (Figs. 168, 169). From this, fine longitudinally arranged strands of collagenous fibers, fibroblasts and fixed macrophages

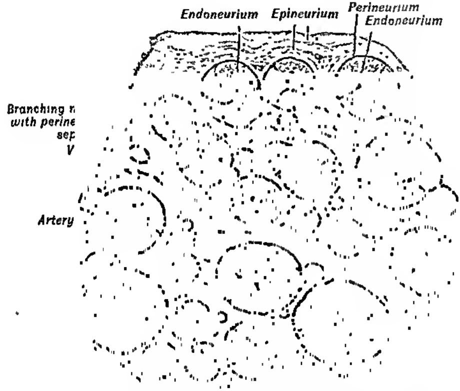

Fig. 169. Portion of a cross section through the sciatic nerve of a newborn. 42 ×. After Schaffer.

pass into the spaces between the individual nerve fibers; this is the *endoneurium.* Where the nerve trunks divide into branches the connective tissue sheaths become thinner. The smaller branches show no epineurium and here the perineurium cannot be distinguished from the endoneurium, being reduced to a thin, transparent, fibrillated membrane covered with flat connective tissue cells resembling endothelial cells (Fig. 170, H) whose outlines can be demonstrated with silver. From this membrane filaments extend to

wrap around each nerve fiber, thus forming the delicate *endoneural* or *connective tissue sheath of Key and Retzius,* a network of elastic fibers attached to the neurolemmal sheath of Schwann. This sheath is also known as the sheath of Henle, although he called it neurilemma. Blood vessels are embedded in the epineurium and perineurium and in the thicker layers of endoneurium.

The following rule on the *functional characteristics of the nerve fibers* holds structure. The fibers of each of these components or systems connect peripherally with similar end organs and centrally with a common type of adjustor, sensory, motor, and the like. Such histologically defined fiber aggregates are therefore functional systems: tactile, gustatory, somatic motor, visceral motor, and so forth. In some, characteristic electrical properties recorded during activity and different from those of other functional fiber systems, have been demonstrated. Although

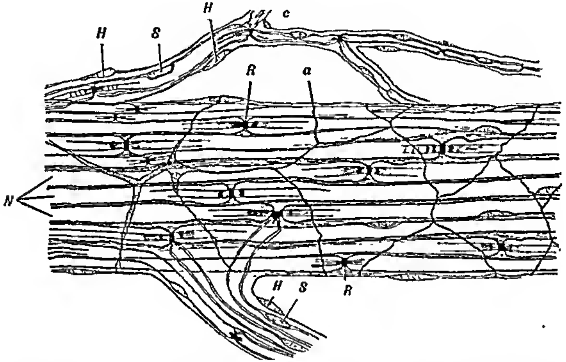

Fig. 170. Nerve trunk of a frog; treated with silver nitrate: *N,* Nerve fibers; *R,* dark crosses at the constriction of Ranvier; *S,* nuclei of Schwann's cells; *H,* nuclei of the cells of the sheath of Key and Retzius; *a,* border lines stained in black between the flat connective tissue cells (perineurium), which surround the entire nerve trunk; *c,* branching of the nerve fiber at a constriction of Ranvier. Redrawn after Ramón y Cajal.

good: the motor nerve fibers of the skeletal muscles are thick and heavily myelinated, those of the smooth visceral muscles are thin and lightly myelinated or without myelin, those of tactile sensibility are of medium size and moderately myelinated, those of pain and taste are thinner, with less myelin or none at all, and those of the olfactory nerve are always unmyelinated. The mixed cranial nerves have been analyzed histologically into *components,* each with fibers of characteristic the analysis is incomplete, the composition of functional components is more evident in the mixed cranial than in the spinal nerves, and is in general the basis of our conception of functional localization in the nervous system.

A particularly clear segregation of functionally different nerve fibers is found in the *spinal roots.* In general, each segmental spinal nerve contains in its ventral roots coarse, heavily myelinated somatic motor fibers for skeletal muscles and thin-

ner, more lightly myelinated or naked visceral motor fibers for the sympathetic nervous system. Its dorsal roots contain cutaneous fibers of several types, as those of deep sensibility, proprioceptive fibers from muscles and tendons, and afferent fibers of visceral sensibility from the sympathetic system. More than half of the dorsal root fibers are naked axons and most of these are distributed with the cutaneous rami. The relative number of myelinated and unmyelinated fibers varies widely in different spinal segments and in the same segment of different mammalian species. In the mixed trunks peripheral to the spinal ganglia the fibers of the motor and sensory roots mingle, and to those are added sympathetic fibers from the communicant rami (Figs. 168, 171). The myelinated fibers of various sizes are readily identified by the clear zones of unstained myelin surrounding the darkly stained axis cylinders. The unmyelinated fibers tend to assemble in small fascicles. Some of these are sensory fibers from the spinal ganglia, others are postganglionic sympathetic fibers.

In the *central nervous system*, in the brain and spinal cord, numerous nerve fibers are also segregated into functional systems. Such are especially the afferent and efferent pathways (cortico-spinal, cortico-nigral, spino-cerebellar, spino-thalamic and many other fiber tracts). Each of these has a special function, partly well known, partly still obscure.

The Peripheral Nerve Endings. Each peripheral nerve fiber, be it sensory, motor or secretory, sooner or later terminates in some peripheral organ with one or several terminal arborizations. Some nerve fibers spread as free endings among the non-nervous tissue cells, others are attached to these by means of complicated structures. The nerve terminals can be divided into two groups according to their main function: (1) *receptors*, which receive sensory impulses either from outside the body or from other tissues, and (2) *effectors*, which transmit impulses to various organs of response, as muscle fibers or glandular cells. The nerve fibers ending as receptors are homologues of dendrites; those with motor or secretory endings are homologues of axis cylinders and their terminations are equivalent to teledendrons. In general, the structure of the nerve endings is adapted to increase the protoplasmic surface of contact between the neuron and its related non-nervous element. The chemical-physical changes which mediate the transfer of the

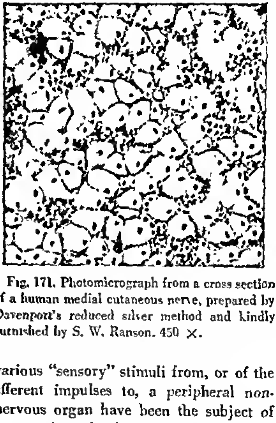

Fig. 171. Photomicrograph from a cross section of a human medial cutaneous nerve, prepared by Davenport's reduced silver method and kindly furnished by S. W. Ranson. 450 ×.

various "sensory" stimuli from, or of the efferent impulses to, a peripheral non-nervous organ have been the subject of intense investigation (see p. 212). According to the tissue, three groups of nerve terminations can be distinguished: (1) endings in muscle, (2) endings in epithelium, and (3) endings in connective tissue.

Nerve Endings in Smooth and Cardiac Muscle. These belong to the unmyelinated or Remak's type of fibers. From complicated plexuses very thin nerve fibers are given off that eventually come in contact with the surface of the muscle cells. Some of these, the sym-

pathetic *motor endings* (Figs. 172, 173), terminate here by means of one, two or more terminal swellings. Possibly, some even penetrate the sub-

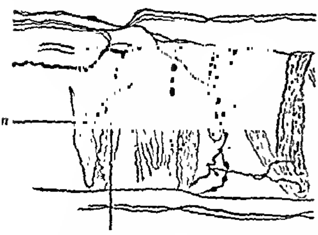

Fig. 172. Nerve endings (*n*) on the smooth muscle cells (*m*) of an artery from the vascular membrane of a rabbit's eye. Redrawn after Retzius.

contact with the muscle fibers themselves. In the cardiac muscle the tissue is permeated by a multitude of thin fibers passing between the muscle trabeculae, on whose surface they form varicosities.

Terminations of the Myelinated Somatic Motor Nerve Fibers on Striated Muscles (Motor Plates). These have a more complex structure (Figs. 174, 175). As the nerve fiber approaches the muscle fiber it loses its myelin sheath. The connective tissue membrane of Key and Retzius with its nuclei extends over the *surface of the sarcolemma and disappears.* The neurolemma or the sheath of Schwann, according to some, also terminates abruptly in the sarcolemma, while, according to others, it may run for a short distance within the plate. At the junction of the nerve and muscle fibers the sarcoplasm forms a mass that varies in form and size beneath the sarcolemma. This is the *motor plate.* It receives the naked axis cylinder which here breaks up into a number of terminal ramifica-

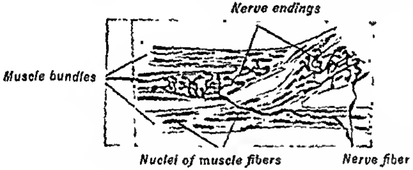

Fig. 173. Smooth muscle spindles in small bronchial muscle bands. Child eight months old. Intravitam methylene blue and borax carmine. Camera lucida. 356 ×. Redrawn after Larsell and Dow.

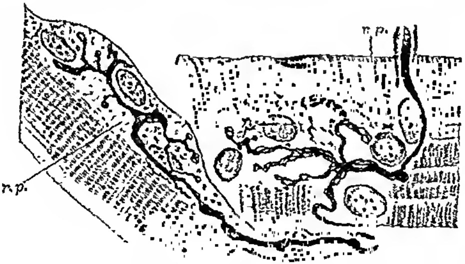

Fig. 174. Motor end-plate from the tongue of the rabbit, showing the "periterminal net" (*r. p.*) of the end-plate. Redrawn after Ramón y Cajal.

stance of the muscle fibers. The *sympathetic sensory fibers* spread in the connective tissue between the smooth muscle bundles or are in

tions. The deep layer of the motor plate adjacent *to the contractile substance is its sole.* Here muscle nuclei may be found in large numbers.

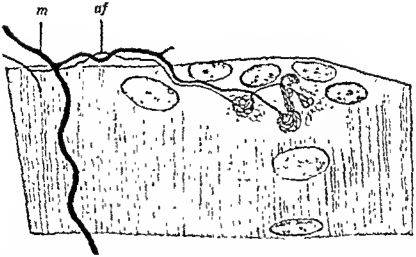

Fig. 175. Motor end plate of a striated muscle from the tongue of a young rat: *m*, Motor nerve fiber; *af*, accessory fiber; "periterminal net" likewise faintly visible. Redrawn after Boeke.

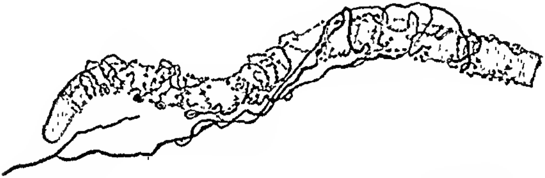

Fig. 176. A sensory nerve ending enveloping a fiber of an ocular muscle. Redrawn after Dogiel.

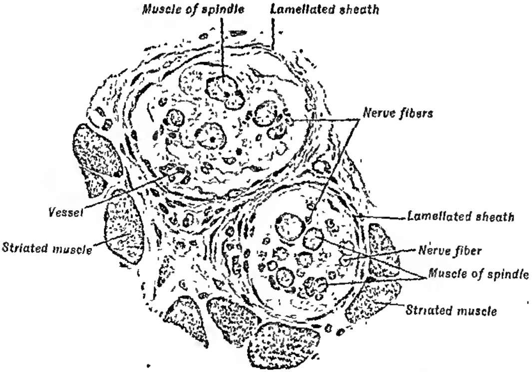

Fig. 177. Cross section of a double muscle spindle from a human tongue. 380 ×. After Schaffer.

pathetic *motor endings* (Figs. 172, 173), terminate here by means of one, two or more terminal swellings. Possibly, some even penetrate the substance of the muscle fibers. The *sympathetic sensory fibers* spread in the connective tissue between the smooth muscle bundles or are in contact with the muscle fibers themselves. In the cardiac muscle the tissue is permeated by a multitude of thin fibers passing between the muscle trabeculae, on whose surface they form varicosities.

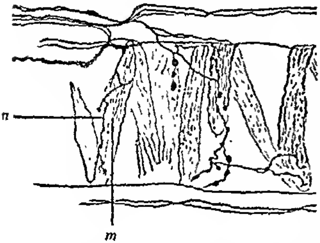

Fig. 172. Nerve endings (n) on the smooth muscle cells (m) of an artery from the vascular membrane of a rabbit's eye. Redrawn after Retzius.

Terminations of the Myelinated Somatic Motor Nerve Fibers on Striated Muscles (Motor Plates). These have a more complex structure (Figs. 174, 175). As the nerve fiber approaches the muscle fiber it loses its myelin sheath. The connective tissue membrane of Key and Retzius with its nuclei extends over the surface of the sarcolemma and disappears. The neurolemma or the sheath of Schwann, according to some, also terminates abruptly in the sarcolemma, while, according to others, it may run for a short distance within the plate. At the junction of the nerve and muscle fibers the sarcoplasm forms a mass that varies in form and size beneath the sarcolemma. This is the *motor plate*. It receives the naked axis cylinder which here breaks up into a number of terminal ramifications. The deep layer of the motor plate adjacent to the contractile substance is its *sole*. Here muscle nuclei may be found in large numbers

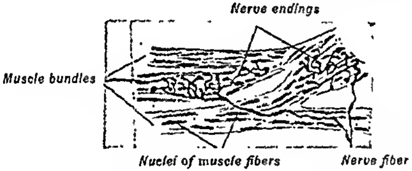

Fig. 173. Smooth muscle spindles in small bronchial muscle bands. Child eight months old. Intravitam methylene blue and borax carmine. Camera lucida. 356 ×. Redrawn after Larsell and Dow.

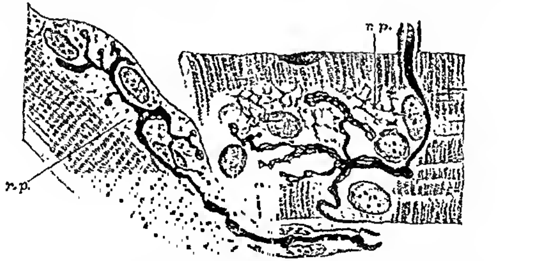

Fig. 174. Motor end-plate from the tongue of the rabbit, showing the "periterminal net" (*r. p.*) of the end-plate. Redrawn after Ramón y Cajal.

Sensory Nerve Endings in Tendons. These are of several kinds, and are also either simple or encapsulated. In simple forms the naked nerve fibers and their branches spread over the surface of the somewhat changed tendon fibers in small treelike figures of different forms (Fig. 179). The composite forms, as the neurotendinal spindles, the organs of Golgi, resemble the neuromuscular spindles and are always found at the very border of the muscular tissue.

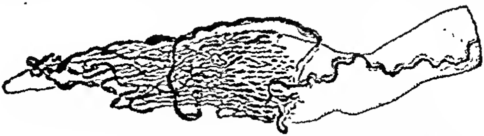

Fig. 179. Sensory nervous apparatus consisting of palisade-like terminal branches, located at the junction of a muscle fiber with a tendon. Redrawn after Dogiel.

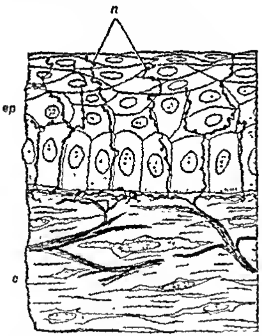

Fig. 180. Free nerve endings (*n*) in the epithelium (*ep*) of a rabbit's cornea; *c*, connective tissue of the corneal substance proper. Impregnation with gold chloride Redrawn after Ramón y Cajal.

The physiological significance of the muscular and tendinous sensory apparatus probably is their responsiveness to various peripheral stimuli of general character, giving sensations of pain, pressure, and particularly of "muscle sense."

Nerve Endings in Epithelial Tissue. These are of both receptor and effector type. Histologically they can be distinguished only in rare instances. The terminations in the epithelial layers of the skin and mucous membranes are regarded as sensory receptors, those in the epithelial glands partly as secretory, partly as sensory. The terminations of the cochlear and vestibular nerves are undoubtedly sensory in their function. The *nervous terminations in glands* (lacrimal, salivary, kidneys, etc.) are all unmyelinated sympathetic fibers forming dense nets on the outer surface of the basement membrane, with branches penetrating the latter and often forming a second network on its inner surface.

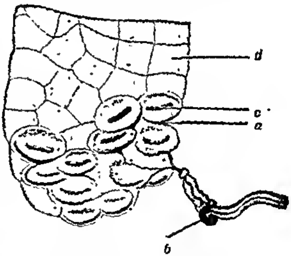

Fig. 181. Merkel's corpuscles in the stratified squamous epithelium (*d*) of the skin of a pig's snout; *a*, tactile disks; *c*, tactile cells; *b*, sensory myelinated nerve fibers. Redrawn after Tretiakov.

They end between the glandular cells as very thin varicose threads.

Free Sensory Epithelial Endings. These are found in the epithelium of the cornea (Fig. 180), epithelium of the mucous membrane of the respiratory passages, skin, and oral cavity, and are especially abundant in places which possess a well developed sensitiveness. In the epidermis these branches do not penetrate farther than the granular layer.

The ramifications of the axis cylinder are accompanied by small, dark nuclei interpreted as those of the neurolemmal cells of Schwann. The

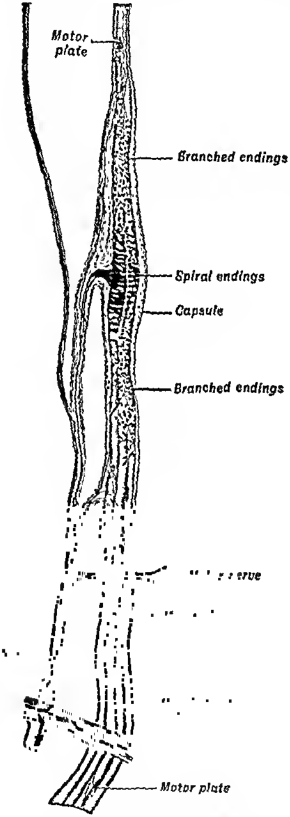

Fig. 178. Neuromuscular spindle of a cat showing nerve endings. Redrawn *after Ruffini*.

terminal arborization of the axis cylinder in a motor plate is beneath the sarcolemma (hypolemmal) and not over it (epilemmal).

The so-called "accessory motor nerve fibers" (Fig. 175, *af*) probably are somatic sensory fibers if not collateral branches of the somatic motor fibers. It is unlikely that they are of sympathetic origin and responsible for the tonus of the skeletal muscles, as has been claimed. On the other hand, the muscles are permeated by a rich sympathetic plexus that is related solely to the blood vessels and connective tissue. The nature of the so-called "periterminal net" of Boeke in the muscle plate (Figs. 174, 175), a structure supposedly bringing about the direct continuity of the neurofibrils and the substance of the muscle fibers, is obscure. Its existence is doubted or denied, or it is considered an artefact by some.

Sensory Nerve Endings in Striated Muscles. These are always present in considerable numbers. Some are located in the muscular tissue, others on tendons or at musculo-tendon junctions. Some terminations are simple, others complex. The interstitial terminations are those distributed in the connective tissue; the epilemmal terminations are those that are in close contact with the muscle fibers but, in contrast with the motor plates, remain on the surface of the sarcolemma. The interstitial terminations may be simple naked branches of the axis cylinders or encapsulated structures. The epilemmal endings likewise may be simple: one or more tortuous axis cylinders, after shedding their myelin sheath at approximately the middle of a muscle fiber, in close contact with the sarcolemma, envelop it by continuous circular and spiral twists. Their varicose twigs terminate with nodular swellings (Fig. 176). More complicated are the so-called *neuromuscular spindles*, found in higher vertebrates only (Figs. 177, 178). They are narrow, long (0.75 to 7 mm. or more) structures slightly thickened in the middle, arranged lengthwise with the bundles of ordinary muscle fibers, and present mainly at the junction of muscles with tendons. Each spindle consists of one or several long striated muscle fibers. They are enveloped by a connective tissue capsule. Each spindle is supplied by thin motor nerves that terminate at the muscle fibers of the spindle with typical motor plates. In addition the spindle is approached by one or more thick sensory nerve fibers. Their axis cylinders, covered with a thin layer of Schwann's cytoplasm and nuclei, wind around the intracapsular portion of the axial muscle fibers and are in close contact with the sarcolemma, forming spirals (Fig. 178). The muscle fibers of the spindles are distinguished by their thinness, abundant sarcoplasm and their peripheral nuclei; in this they resemble the so-called "red muscle fibers."

in the depressions between its papillae, in the external root sheath of the hair, and elsewhere.

Nerve Endings in Connective Tissue. These are numerous and of many forms, particularly in the derma, under the epithelium and mesothelium of the mucous and serous membranes, around the joints, in the endocardium and elsewhere. The terminations of the somatic cerebrospinal nerve fibers in the connective tissue are either free or encapsulated endings, or are connected with special tactile cells of epithelial origin. More complex endings are in the skin and hypodermis, in mucous and serous membranes, endocardium, cornea, sclera, periosteum, and elsewhere. *Nonencapsulated nerve glomeruli* are frequent in the papillary layer of the skin, in the connective tissue of the mucous membranes as that of the urinary bladder, in the peri- and endocardium, periosteum, and so forth. In these the terminal branches of the nerve fibers form spherical or elongated structures resembling glomeruli.

Encapsulated Terminal Sensory Apparatus. In these there is a special connective tissue capsule of varying thickness surrounding the actual nerve endings. The capsule attains its greatest thickness in the *corpuscles of Vater-Pacini* (Fig. 182). Terminations of this type are found in the deeper layers of the skin, under the mucous membranes, in the conjunctiva, cornea, heart, mesentery, pancreas, and in loose connective tissue in general. The size of these structures is considerable (1 to 4 x 2 mm.) and they are white. Each corpuscle is supplied with one or more thick myelinated fibers which lose their myelin. Their sheaths of Schwann and of Key-Retzius are continuous with the capsule. Of the same type are the so called *genital corpuscles found in the skin of the external genital organs* and of the nipple. *Meissner's corpuscles* (Fig. 183) are found in the connective tissue of the skin of the palms, soles, and tips of the fingers and toes. They are elongated, pear-shaped or elliptical formations with rounded ends, located in the cutaneous papillae, with the long axis vertical to the surface. Their size varies (40 to 100 $\times$ 30 to 60 μ). The *corpuscles of Golgi-Mazzoni or the terminal bulbs of Krause* are similar in structure to the corpuscles of Vater-Pacini but are smaller in size and simpler in construction (*On terminations of the dendrites* and axis cylinders in the brain and spinal cord, see p. 209.)

THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system is composed of numerous small ganglia, some of which are arranged in two chains along the spinal column, and more of which are scattered among other tissues of the body, all being connected by an intricate system of nerve fibers. The autonomic nervous system consists of the *parasympathetic* (*craniasacral*) and *sympathetic* (*thoracolumbar*) outflows.

The sympathetic trunks and the ganglia contained within them, *the vertebral ganglia*, are the chief avenues of communication between the central nervous system and the outlying sympathetic ganglionic plexuses. Each sympathetic trunk contains ganglia at the level of exit of most of the spinal nerves. The *communicating branches* (rami communicantes) pass between the trunk and the spinal nerves in these regions.

All the neurons of the central and the peripheral systems primarily concerned with the regulation of visceral activities form the autonomic portion of the *visceral nervous system*. On the sensory or afferent side of the reflex arcs, all neurons whose peripheral processes extend from the viscera through the communicating branches to the spinal ganglia are not strictly autonomic, but belong to the somatic nervous system. Neurons which lie wholly within the sympathetic system may exercise a local regulatory control over the viscera to which they are related. These local adjustors are under modification by the visceral centers of the spinal cord and brain.

The *visceral sensory neurons* cannot be readily distinguished from the somatic sensory or craniospinal elements, with which they are mingled in the spinal ganglia, except by following their peripheral fibers outward into the communicating branches. The bodies of the *visceral motor neurons* of the spinal nerves are segregated in the "intermedio-lateral gray column" of the spinal cord (Fig. 184, *int. lat.*). Their axons pass out of the cord into the ventral roots and through

Fig. 182. Cross section of a corpuscle of Vater-Pacini, from the derma of the sole of a human foot: *DL*, Lamellae; *G*, blood vessel between the superficial lamellae; *IK*, inner bulb. 110 ×. After Schaffer.

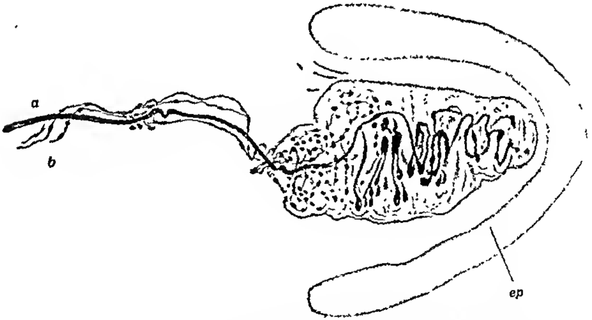

Fig. 183. Meissner's corpuscle of a dermal papilla of a human finger: *a*, Thick and, *b*, thin myelinated fiber; *ep*, epithelium. Methylene blue. Redrawn after Dogiel.

Merkel's Corpuscles. These (Fig. 181) consist of a modified epithelial cell (tactile cell) and of a peculiarly constructed terminal widening of the axis cylinder, the tactile disk or meniscus. Such corpuscles, usually in groups, are found in the deep layers of the epithelium of the skin.

pathetic and parasympathetic systems as functionally antagonistic systems on the basis of different reactions to certain drugs no longer holds.

(1) *The cranial autonomic* (*cranial parasympathetic system*) includes the preganglionic neurons whose axons enter the oculomotor, facial (chorda tympani), glossopharyngeal, vagus and accessory nerves, and also the postganglionic neurons of the peripheral ganglia in the head and trunk. (2) *The sacral autonomic* (*sacral parasympathetic system*) includes the preganglionic neurons whose axons emerge in the ventral roots of the second to the fourth sacral spinal nerves, and also the related postganglionic neurons of their peripheral sympathetic ganglia. (3) *The thoracolumbar autonomic* (*sympathetic system*) includes preganglionic neurons whose cell bodies lie in the intermediolateral gray columns of the thoracolumbar portion of the spinal cord and whose axons emerge in the ventral roots of the thoracic and the first three or four lumbar nerves. and the related postganglionic neurons.

Sympathetic Nerve Cells. The cell bodies of the preganglionic visceral efferent neurons are small, spindle-shaped elements in the intermediolateral gray column of the spinal cord.

The postganglionic neurons of the craniosacral visceral nervous system lie, as a rule, close to the viscera innervated. The preganglionic fibers, accordingly, are relatively long—as in the vagus nerve—and the postganglionic fibers are short. On the other hand, most of the synapses of the thoracolumbar system are in the ganglia of the sympathetic chains or trunks and therefore their postganglionic fibers are relatively longer.

The nervous elements of the sympathetic ganglia are generally small and have such diverse shapes and structure that some maintain that no morphological classification is practicable. The cells are generally multipolar, with the dendrites and axon sometimes being clearly distinguishable, in other cases showing no obvious difference. For a typical example see the description of the postganglionic neurons of the intestine (p. 201).

The cell body may be surrounded by a capsule of satellite cells, which, like those of the craniospinal ganglia, are ectodermal elements related to the cells of Schwann in the nerve sheaths. In the outlying sympathetic ganglia these capsules may be absent, but the cells of Schwann accompany the peripheral sympathetic fibers everywhere.

NEUROGLIA

The term neuroglia is applied to the following interstitial tissues: the *ependyma* which lines the ventricles of the brain and spinal cord, *neuroglial cells* and their expansions or "fibers" which bind together the neurons in the central nervous system and in the retina, and the *satellite* or *capsular cells* of the peripheral ganglia. The *cells of Schwann* of the peripheral nerves may be considered as equivalent to peripheral neuroglia.

Ependyma. In the early embryonic stages of the brain and spinal cord the wall of the neural tube is a simple epithelium (Fig. 196). Certain thin nonnervous parts of the brain retain this structure throughout adult life, as the epithelial layer of the chorioid plexus (Fig. 204). In most other parts of the neural tube, the wall is greatly thickened by the differentiation and multiplication within it of neurons and neuroglial elements. The lining of the inner surfaco of the wall enclosing the ventricular cavities always retains an epithelial character (Fig. 185). This lining membrane, the adult ependyma, is composed of the inner ends of the persisting epithelial cells, with their nuclei and some of their cytoplasm, and such derivatives of the primitive embryonic epithelium as remain in connection with it.

The embryonic ependyma is ciliated and in some parts of the ventricular lining the cilia may persist in adult life. In the mature brain, their broad bases taper to long, threadlike processes that may branch and that are lost among other elements of the brain (Fig. 185). In a few places, where the nervous wall is thin, as in the ventral fissure of the spinal cord, some ependymal cells span the entire distance between the ventricular and external surfaces (Fig.

the white communicating branches, to end either in a vertebral ganglion of the sympathetic trunk or in one of the outlying ganglionic plexuses (a prevertebral gaoglion). These axons, *preganglionic fibers*, with thin or no myelin sheaths, always terminate in a sympathetic ganglion. Here they effect synaptic junction with secondary visceral motor neurons, whose axons the preganglionic efferent neurons, while the gray contain unmyelinated axons of the postganglionic efferent neurons which carry visceral efferent nervous impulses from the sympathetic ganglia to the spinal nerves. Among the latter are the *vasomotor fibers* going chiefly to arterial muscles, the *pilomotor fibers* to the small muscles of the hair follicles, and the *sudorif-*

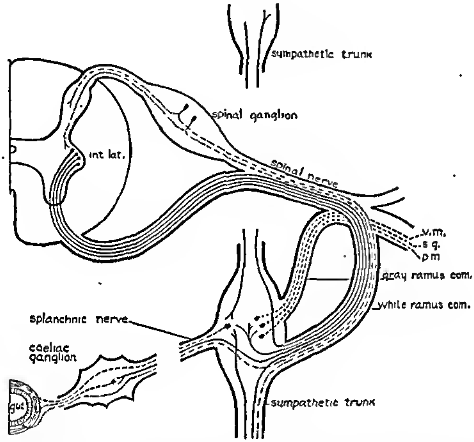

Fig. 184. Diagram of the relations of the sympathetic trunk to the spinal cord and spinal nerves. Visceral sensory fibers are drawn in dot-and-dash lines, preganglionic fibers in continuous lines, and postganglionic fibers in broken lines. For clearness the rami communicantes are drawn farther separated from the spinal ganglion than natural: *int. lat.*, The intermediolateral gray column of the spinal cord, axons of whose cells form preganglionic fibers; *p.m.*, pilomotor postganglionic fiber; *s g.*, postganglionic fiber for sweat glands; *v.m.*, vasomotor postganglionic fiber. From Herrick's Introduction to Neurology.

—the mostly unmyelinated *postganglionic fibers*—transmit the impulse to visceral muscles or glands.

The *communicating branches* (Fig. 184) are the paths of connection between the spinal nerves and their adjusting centers in the spinal cord on one hand, and the local visceral adjusting mechanisms on the other. The white branches contain myelinated fibers of the sensory and of

erous fibers to the sweat glands. Other postganglionic fibers go to the viscera in the sympathetic nerves, like the splanchnic nerves, where they are mingled with the myelinated axons of the preganglionic and visceral sensory neurons.

The efferent part of the *autonomic system* can be subdivided into three sections on an anatomical basis. However, the distinction frequently made between the sym-

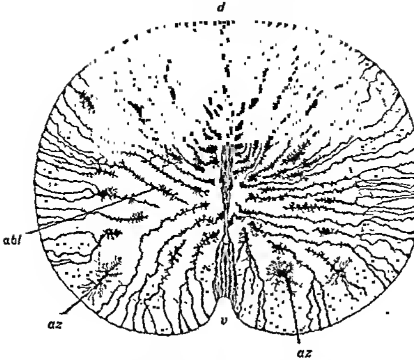

Fig. 187. Cross section of the spinal cord of a newborn mouse: v, Ventral side; d, dorsal side; *abl*, astroblasts moving away from the central canal; they are *transformed into stellate neuroglia* cells (astrocytes), *az*. Method of Golgi. Redrawn after Ramón y Cajal.

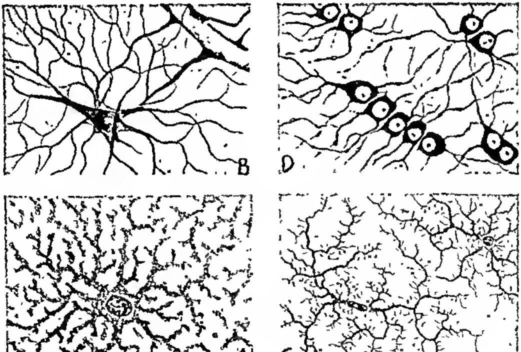

Fig 188. Neuroglial cells of the cent--- --- *B*, fibrous astro-cyte; *C*, microglia; *L*

These cells also are often attached to the blood vessels by means of their processes. The protoplasmic astrocytes are found chiefly in the gray substance, the fibrous astrocytes in the white substance of the brain insinuated between the fascicles of nerve fibers. Mixed or *plasmatofibrous astrocytes* are occasionally encountered at the boundary between the gray and white sub-

187). All of them do so in the early embryonic stages (Fig. 186). In these cases the ependymal *cells form a dense internal limiting membrane* at the ventricular end.

At the external surface under the *pia mater* the ependymal threads and bars expand into pedicles

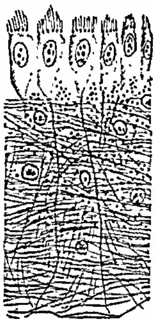

Fig. 185. The neuroglia from the ependymal layer of the fourth ventricle (tuberculum acusticum) of a cat, with ciliated ependymal cells. Redrawn after Rubaschkin.

which fuse into a thin, smooth and dense membrane, the *external limiting membrane* of the central nervous system (Figs. 186, 187). Similar membranes are formed around the blood vessels (Figs. 188, *B*; 189). In most parts of the adult human brain and spinal cord, with the increase in the thickness of the wall, the ependymal threads are stretched between the internal and external limiting membranes beyond the breaking point. Finally the ependymal threads lose contact with the opposite face of the wall, remaining connected with their own ependymal cells (Fig. 187).

Neuroglia Proper or "Glia." In any section of the central nervous system prepared by ordinary histological methods, small nuclei are seen scattered among the nerve cells and their processes (Figs. 191, 203). The cytoplasm and long processes of these neuroglial elements are revealed by special histological technic.

Three types of neuroglia are distinguished: *astrocytes, oligodendrocytes* and *microglia.* The first two are undoubtedly of ectodermal origin, as are the nerve cells proper. The third, or microglia, according to Río-Hortega, originates from mesodermal cells of the pia mater which migrate into the central nervous system along the blood vessels. His opinion is not accepted by all.

The *astrocytes,* termed also "astroglia," "macroglia" or "spider cells," are of two varieties. The first is the *protoplasmic astrocyte* with nucleus larger than in oligodendrocytes and microglia, and with relatively abundant granular cytoplasm and numerous rather thick plasmatic expansions (Fig. 188, *A*). Many of their processes are attached to the blood vessels and to the pia mater by means of expanded pedicles. In other cases, the body of the cell lies directly on the wall of the blood vessel or on the inner surface of the pia. Some of the smaller cells of this variety lie close to the bodies of the neurons and are

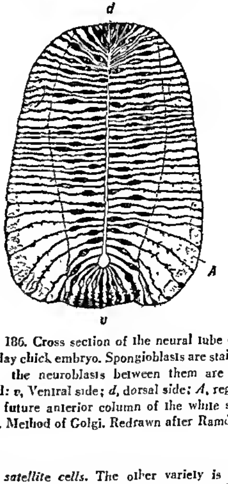

Fig 186. Cross section of the neural tube of a three-day chick embryo. Spongioblasts are stained black; the neuroblasts between them are unstained: *v*, Ventral side; *d*, dorsal side; *A*, region of the future anterior column of the white substance. Method of Golgi. Redrawn after Ramón y Cajal.

called *satellite cells.* The other variety is the *fibrous astrocyte* (Fig. 188, *B*), distinguished from the first by long, relatively thin, smooth, and little branched expansions. Embedded within the cytoplasm of their bodies and expansions are fibrillar structures or *neuroglial fibers* (Fig. 190).

processes and the body do not appear smooth but are covered with a considerable number of tiny pointed twigs or "spines." The microglial cells are scattered everywhere throughout the brain and spinal cord.

The neuroglia of the adult central nervous system develops from the primitive spongioblasts of the embryo (Fig. 195), and is of ectodermal origin. An exception is the microglia which possibly is mesodermal. In the mature brain and spinal cord the neuroglial tissue as a whole forms an extremely complicated supporting framework of cells and their expansions, with a multitude of passages, in which the nerve cells proper or neurons and their processes are suspended. Like the nerve cells, the supporting neuroglial cells do not form an actual syncytium (as assumed by some), but they, too, retain a certain degree of individuality, although apparently less than in the case of the neurons, as the adjoining neuroglial cells form virtually a sealed honeycomb. In the chambers of this honeycomb the nerve cells and their expansions are individually encapsulated and thus separated or insulated from one another (Fig. 190). Only at the points of the synapses are the neuroglial barriers broken and only here is a direct contact between the neurons possible. The essentially insulating function of neuroglia and of Schwann's cells is likewise attested by their participation in the formation of myelin sheaths in those axons where a greater speed of transmission is attained. This insulating rôle of the neuroglia, besides the relative functional and trophic independence of the neurons, enables each neuron to function in its own specific way.

The neuroglia appears also to be an important mediator for the normal metabolism of the nervous elements proper, although little is known in this respect. More is known about the activity of the neuroglia in pathological processes. Whenever the neurons are affected by a local or distant pathological process, the surrounding neuroglial elements always react in some way. They are actively involved in the degeneration and regeneration of the nerve fibers, in vascular disorders, in various infectious processes, and are one of the chief sources of tumors of the central nervous system. The fully developed, mature neurons of higher vertebrates, except those that form parts of the peripheral nervous system, have practically lost the ability to divide, which they possessed as embryonic neuroblasts, and their ability to regenerate is greatly reduced or, as in the brain, entirely lost. On the other hand, the satellites, neurolemmal cells and neuroglia fully retain this ability and participate in all reparative reactions after injuries to the nervous system. In particular, the microglial cells assume in such cases a great variety of forms, with active migration and phagocytosis. They probably play a rôle in the metabolism of the nerve cells, and phagocytose disintegrating nervous elements (p. 222).

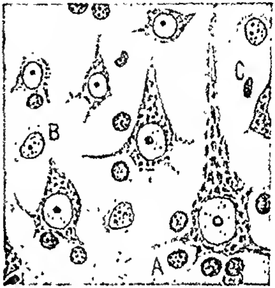

Fig. 191. Portion of human cerebral cortex stained by the method of Nissl. *A*, Naked nuclei of oligodendroglia cells; *B*, protoplasmic astrocyte; *C*, nucleus of microglia cell. In addition, large and small pyramidal neurons. After del Río-Hortega.

THE SYNAPSE AND THE INTERRELATIONSHIPS OF NEURONS

Essentially, the nervous system is composed of complex chains of neurons so arranged as to permit the passage of nervous impulses from one neuron to other neurons in the central nervous system and from the non-nervous to the nervous organs and vice versa. The place of contact where the substance of the axon ending of one neuron meets the body or the dendrites of its related neuron is called a *synapse*.

It is usually assumed that, whenever a neuron is activated by an extraneous impulse, the impulse spreads over all of its parts—the perikaryon, the dendrites and the axon. Yet physiologically, the excitation passes only from the dendrites or body, where activation normally occurs, to the axis cylinder and its endings. This

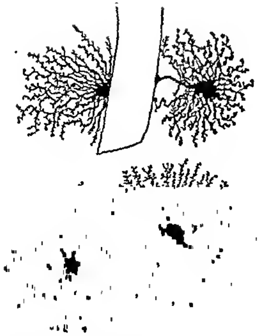

Fig. 189. Short-rayed astrocytes from the gray matter of the brain of an adult man; the two upper ones are connected with the walls of a blood vessel by their processes. Golgi method. Redrawn after Ramón y Cajal.

while those that pass into the white substance are fibrous.

The *oligodendrocytes*, also called "oligodendroglia" and "oligoglia" (Fig. 188, *D*), are closely akin to the astrocytes which they resemble in most respects. They are smaller and have smaller nuclei, although there are many transitional forms. The name is derived from the fact that their few and slender processes have few branches. The processes rarely if ever form footlike expansions on blood vessels and no true neuroglial fibers are related to them. They seem to be in an especially intimate relationship with the nerve fibers along which they are frequently found in rows or columns. Because of this they are regarded as the central homologue of the *neurolemmal cells of Schwann. In the gray sub*stance those oligodendrocytes that adjoin the nerve cells proper are called "satellites" (Fig. 191, *A*).

In the *microglia* or Hortega's cells (Fig. 188, *C*), the nucleus is likewise small but deeply stained and surrounded by scanty protoplasm. The few expansions are rather short and, unlike

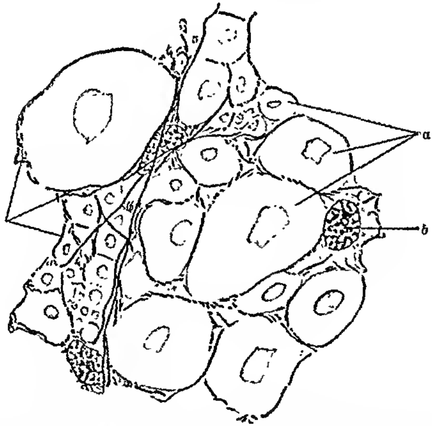

Fig. 190. Cross section of the white matter of the spinal cord of a cat stained for neuroglia fibers: *a*, Nerve fibers of different calibers, with a centrally located axis cylinder and with a wide, pale myelin sheath; *b*, neuroglia cell; *c*, neuroglia fibers. Weigert method. 1000 ×. (A.A.M.)

stance; those of their processes that spread into ... substance have a protoplasmic character,

the more or less straight expansions of the astrocytes, are twisted in various ways. Also, the

processes and the body do not appear smooth but are covered with a considerable number of tiny pointed twigs or "spines." The microglial cells are scattered everywhere throughout the brain and spinal cord.

The neuroglia of the adult central nervous system develops from the primitive spongioblasts of the embryo (Fig. 195), and is of ectodermal origin. An exception is the microglia which possibly is mesodermal. In the mature brain and spinal cord the neuroglial tissue as a whole forms an extremely complicated supporting framework of cells and their expansions, with a multitude of passages, in which the nerve cells proper or neurons and their processes are suspended. Like the nerve cells, the supporting neuroglial cells do not form an actual syncytium (as assumed by some), but they, too, retain a certain degree of individuality, although apparently less than in the case of the neurons, as the adjoining neuroglial cells form virtually a sealed honeycomb. In the chambers of this honeycomb the nerve cells and their expansions are individually encapsulated and thus separated or insulated from one another (Fig. 190). Only at the points of the synapses are the neuroglial barriers broken and only here is a direct contact between the neurons possible The essentially insulating function of neuroglia and of Schwann's cells is likewise attested by their participation in the formation of myelin sheaths in those axons where a greater speed of transmission is attained. This insulating rôle of the neuroglia, besides the relative functional and trophic independence of the neurons, enables each neuron to function in its own specific way.

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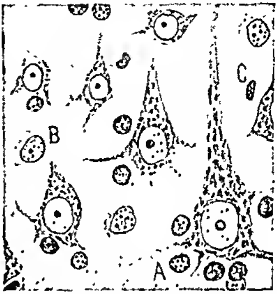

Fig 191. Portion of human cerebral cortex stained by the method of Nissl. *A*, Naked nuclei of oligodendroglia cells; *B*, protoplasmic astrocyte; *C*, nucleus of microglia cell. In addition, large and small pyramidal neurons. After del Río-Hortega.

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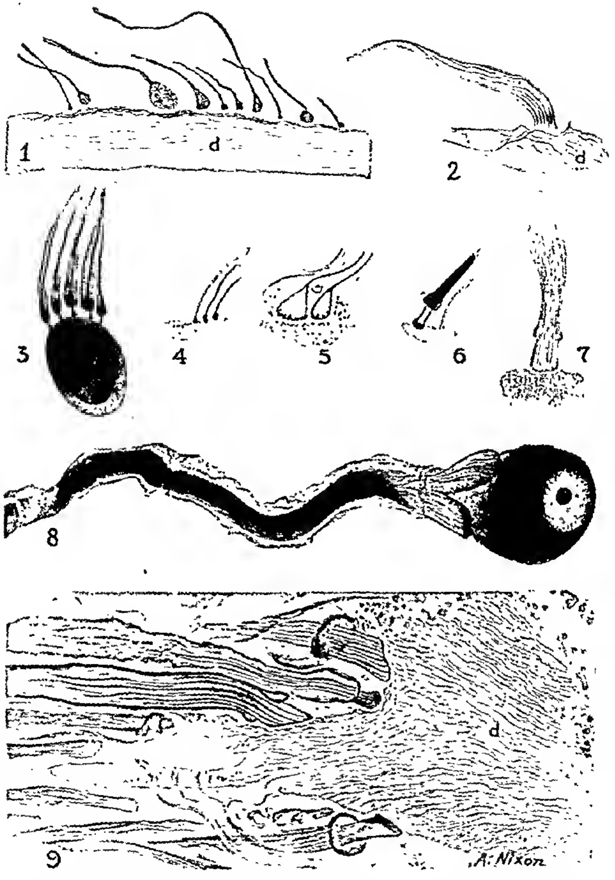

Fig. 192. Types of nerve fiber endings in the vertebrate central nervous system. *1*, Terminal enlargements (end-feet of Held) of unmyelinated nerve fibers which end in relation to dendrite of another nerve cell (*d*). *2*, Club ending (of Bartelmez) of large nerve fiber which ends abruptly on dendrite of another nerve cell (*d*). Note arrangement of neurofibrils in the two synaptic elements. *3*, Fine myelinated fibers ending on nerve cell by means of tiny "end-feet." *4*, Similar endings of

means that there is a *functional polarization of the synapse* which permits the nervous influence, whatever its intimate nature, to pass only from the axon of one neuron to the dendrites or body of the next neuron in the chain. Without this polarization of the synapse, and consequently of the neuron as a whole, nervous impulses would spread in all directions in the central nervous system and specific modes of reaction would be impossible.

What the basis of functional polarization at the synapse, leading to irreciprocal conduction across it, may be is not certain. There is much coordinated physiological and anatomical evidence, however, that this may be in part a matter of geometric relations. A small fiber, for example, may be able to excite a large one, where the reverse is impossible.

A great mass of physiological and morphological data makes it seem certain that the two sides of the synaptic membrane are dissimilar in their minute structural organization and in their functional properties. Of particular interest here are the giant terminal feet or "club endings" measuring up to 7 microns across (Fig. 192; Bartelmez, Bartelmez and Hoerr, and Bodian). In each of these, neurofibrils terminate close to the synaptic boundary with button-like swellings and do not pass into the related neuron.

The number of synapses on a neuron may vary from only a few to as many as 1800 on the body of a single motor neuron. The forms of the synapses vary in the extreme (Fig. 192). Usually they are tiny swellings at the ends of the axon endings. Or the twigs form bouquets or loose baskets, and the like, adhering to the body or dendrites of another nerve cell. Each variety of neuron is distinguished by its own form of synaptic terminations, some having endings of several kinds. Neurons that have no direct or even indirect synaptic relationship with one another are independent of one another. Bodian (1942) has an excellent review on the "Cytological Aspects of Synaptic Function."

Much of the capacity of the nervous system is due to the great variety of neurons and their structural and functional specialization. The unity of the nervous system as a complex whole is maintained by the countless synaptic connections among its constituent cells, and perhaps also by operation of potential fields and electric currents flowing between cell groups. However, in those viscera which always act *in toto* and where there is no minute territorial delimitation of function, the nerve cells or their expansions perhaps undergo such intimate connections with one another as practically to abolish most visible boundaries such as are found in the other parts of the nervous system. This problem needs further investigation.

The conception of the structure of the nervous system sketched above forms the almost universally held *neuron doctrine.* It maintains that each mature nerve cell represents a cellular unit capable, under

unmyelinated fibers on dendrite of another nerve cell (compare with the four smallest endings in *1*). *5,* Large "end feet" on dendrite of another nerve cell. Note band of red-stained granular mitochondria at the terminal surfaces of the "end feet." Compare with largest end-feet in *1*. *6,* Clublike ending of small myelinated fiber, and *7,* clublike ending of large myelinated fiber, ending on dendrite of another cell. Neurofibrils in dendrite of *7* are cut transversely and appear as fine dots. Note myelin sheath (red) investing axon and compare with *2* in which myelin is unstained. *8,* Large myelinated fiber (left) forming a calyciform ending, embracing portion of another nerve cell (right). Note band of granular mitochondria at terminal surface of calyx, and myelin sheath (red) enveloping axis cylinder (left). *9,* Several large myelinated axons (left) ending by means of club-shaped terminals on dendrite (*d*) of large nerve cell. Note arrangement of neurofibrils in axons and in dendrite upon which they terminate. *1* and *2* stained for neurofibrils with reduced silver *3–9* fixed by injecting Zenker-formol into blood vessels of living animal; silver followed by Mallory-azan stain. All from the brain of the goldfish, and drawn at 1410 ×. After Bodian, 1937. Courtesy of Wistar Press.

given circumstances, of independent existence. The processes of a nerve cell are dependent on the body with its nucleus; when cut off they die, although peripheral processes may regenerate from the perikaryon (p. 220). The body and nucleus of the nerve cell are the trophic center of the whole neuron. The connections between different neurons are so polarized marized, as of 1946, by Eccles and by Gerard. At present, the most that can be said is that the neurons influence one another through the synapses. It should be pointed out that much of the controversy over the neuron doctrine was due to the assumption that crude histological methods can give evidence on such delicate cytological structures as the synapses in

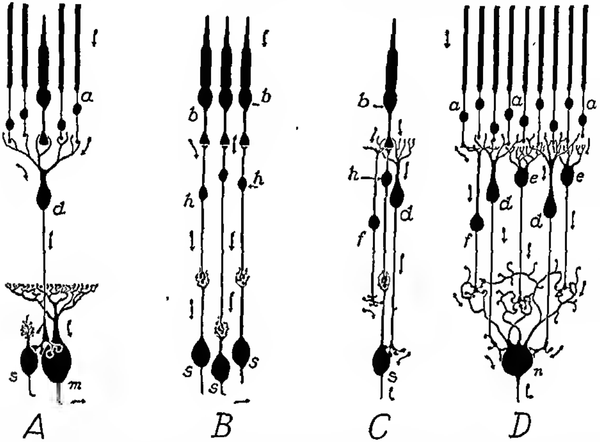

Fig. 193. Several types of synaptic relationships of neurons: *A*, *d*-Bipolar neuron serving as a common pathway for both rods and cones (a); *B*, isolated conduction or one-to-one relationship of neurons (*b-h-s*); *C*, a single excitation (in *b*) is transferred to each of the three related neuron varieties (*d*, *f*, *h*); *D*, excitations from a number of rods (*a*) pass through intermediary neurons (*d*, *e*, *f*) to a single large neuron (*n*). Examples from the primate retina (see Fig. 539). Courtesy S. Polyak.

that conduction of nervous impulses is always from the axon of one neuron to the dendrites or body of the next. If a nerve cell suffers irreparable injury, adjoining nerve cells are not necessarily affected.

Various theories based on chemical and electrical changes observed in nerves have been advanced to explain the transmission of the nervous impulse from one neuron to another. These have been well sum- the central nervous system and the relationships of peripheral nerves to end organs. As finer methods are developed we shall be able more adequately to analyze the submicroscopic structure of neurons and their synapses, for there is much physical evidence that a thin outer layer (100 to 200 A) of the nerve cell is a critical zone in the transmission of the impulse.

Examples of Interrelationships of Neurons. Except in the primate retina, where one-to-one synapses are found (Fig. 193, *B*), practically all neurons are connected with several or many other neurons. With the aid of the Golgi impregnation and other methods, several different types of relationships between neurons have been shown to exist. These vary from extremely complex relationships involving the processes of hundreds of cells to relatively simple configurations. It will suffice here to give examples of a few extreme categories. For instance, attached to the body and dendrites of many large motor cells of the anterior gray columns of the spinal cord are many hundreds of synaptic buttons of axon endings of neurons in the cerebral cortex, the cerebellum, correlation cells of the spinal cord, the motor and similar centers of the brain stem, and in the periphery. The spinal motor cells serve, accordingly, as the *final common pathway* where the nervous impulses from a variety of sources are transmitted to effector organs. A clear instance of this is found in the giant Mauthner's cells in the medulla oblongata of fishes (Fig. 192). In the retina, the *d*-bipolars serve as a common pathway for impulses from both rods and cones (Fig. 193, *A*). The reverse arrangement is shown in Fig. 193 *C*, where one retinal cone is in contact with three neurons (*d*, *f*, *h*).

In the frequent arrangement in which a few neurons are related to a large group of neurons, the reaction is not commensurate with the initial stimulus, but is determined by the number and kinds of reacting neurons, often arranged in internuncial chains effecting inhibition or facilitation of the impulse (Lorente de Nó). Thus, in a spinal reflex arc (Fig. 194) the excitation of a few peripheral sensory elements may activate a great number of motor neurons, and the total response or effect may exceed many times the energy that initiated it. Another example is the excitation of a few photoreceptor cells of the retina and the subsequent turning of the eyes and head toward the source of the stimulus.

These glimpses of the exceedingly intricate interconnections between neurons, coupled with their enormous numbers (it is estimated there are 9,200,000,000 neurons in the cerebral cortex alone), and their extreme variability in the various parts of the nervous system, indicate the extreme complexity of structure and function.

White Matter Conducts, Gray Matter Integrates Impulses. The white matter of the brain, the numerous tracts of the brain stem and spinal cord, and practically all peripheral nerves, are chiefly or entirely made up of myelinated or unmyelinated axis cylinders. These parts serve, accordingly, to transmit nervous excitations from the viscera to the central nervous system, or vice versa, or from one part of the brain or cord to other parts. There is no evidence that any essential modification of the passing excitations occurs in the fibrous parts, except such as might be due to a decrease of intensity where the axis cylinders divide into a number of branches.

In the regions composed chiefly of nerve cells, unmyelinated and some myelinated nerve fibers, in the nerve centers which constitute the so-called "gray matter" (cerebral and cerebellar cortex, various subcortical nuclei, gray columns of the spinal cord, peripheral ganglia), the situation is reversed. Here innumerable reciprocal contacts between the various types of neurons make possible an endless variety of mutual influences. It is here that the centralizing, selecting, combining, dividing, and intensifying of incoming impulses is performed and the resulting impulses sent back to the peripheral organs of execution. A preparation of such an area shows the bodies of the cells arranged in a certain order, usually in

layers. The space between the cellular layers, and also between the individual cells, is filled with innumerable axis cylinders and dendrites, and also with neuroglia and blood vessels. The nervous expansions usually are without myelin sheaths, which accounts for the gray appearance of these parts in fresh condition.

When stained with routine methods, the nervous plexiform substance between the cell bodies has a dotted aspect, and was often called *neuropil.* Where the separation of cellular elements from the plexiform substance is complete (as in the molecular

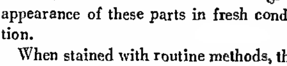

Fig 194. Diagram illustrating the simplest spinal reflex arc consisting of two nervous elements or neurons, a sensory neuron connected with the skin and a motor neuron connected with a muscle. Physiologic connection between the two neurons is effected within the spinal cord. Modified from van Gehuchten.

layer of the cerebellar cortex, in the plexiform layer of the cerebral cortex, and in the retina) certain layers are composed almost exclusively of the naked expansions of the neurons and of the neuroglia. Since in these layers huge numbers of synaptic contacts take place, they can be called *synaptic fields* (layers 5 and 7, Figs. 534, 539) (cf. Herrick, 1934).

The pattern of the cell and fiber arrangement in the gray nervous substance varies much in detail from place to place. Every subcortical nucleus, peripheral ganglion, and locality of the cerebral cortex has architectural features of its own. Thus the cortex in the precentral convolution of the primate brain, which coincides with the so-called *motor area*, differs considerably from that of the postcentral convolution where the conscious *somatosensory function* is represented, or from any other portion of the cerebral cortex. One of the most characteristic cortical areas is along the calcarine fissure of the occipital lobe which corresponds with the *visual center* (area striata). Another, in the Sylvian fossa, is the *auditory center.* Numerous other localities with characteristic histological structures, are connected with particular functions. Some functions of the central nervous apparatus are territorially well localized, whereas others are properties of large volumes of tissue.

The gray nervous substance differs profoundly from the simple nerve fibers or axons in several respects. Although most reflexes are transmitted over several intercalated neurons, a diagrammatically oversimplified reflex arc may serve as an example. It can be conceived to be composed of a sensory (afferent) and a motor (efferent) neuron synaptically interlocked in the nervous center, together with the corresponding peripheral receptor and effector (Fig. 194). Such a mechanism is functionally characterized by the following: (1) It fatigues rapidly in contrast to the simple nerve fibers which are exhausted slowly—or never completely as the myelinated fibers; (2) the reflex is blocked in the center by a fraction of the amount of a drug which suffices to block the peripheral nerve fiber; (3) the direction of the excitation is always from the sensory fiber to the motor or secretory fiber, indicating the functional polarity of the gray substance, termed irreversibility or irreciprocal conduction; (4) the response varies greatly with respect to the latent period and the intensity, depending on various conditions of the central nervous system itself; this is termed variabil-

ity; (5) the latent period is much longer than in the nerve fiber, and there may be an after-discharge, that is, the response may continue for some time after the stimulus ceases; (6) whereas one or a few stimuli may have no effect, an effect may result from numerous stimuli applied in sequence, which indicates summation; (7) certain nerves are capable of slowing down or stopping the reflex response induced by the stimulation of other nerves, an effect interpreted as inhibition; (8) the rhythm of the response in a reflex is usually slower than that of the applied stimulus (Sherrington. For further details see Gerard, 1931).

Little is known of the mechanism underlying these phenomena. Some of the properties of the gray nervous substance can be attributed in part to the synapses as such. They alone may be responsible for the functional polarity of the gray substance. Some other features, too, may be due to difference in size, number of contacts and minute structure and organization of the various types of synaptic junctions.

Electric Manifestations of the Brain. Considerable attention has been paid to two kinds of electric activity of the cortical gray substance. The spontaneous potentials manifest themselves by automatic rhythmic "beats" even in the absence of outside impulses; the other, the evoked potentials, often likewise rhythmic, arise only when a peripheral sensory organ is stimulated or a motor action initiated. These potential changes often have some characteristic local features limited to a particular architectural area. The subcortical pathways, various nuclei, the spinal cord and even the peripheral ganglia may show a like activity.

It would thus seem that the normal cerebral cortex is in a state of constant activity, irrespective of the stimuli from the peripheral sense organs. This is paralleled by its high metabolism which, for oxygen, has been shown to be 25 times as great as in muscle or peripheral nerve. A great deal of automatic rhythmic activity seems to be inherent even in single neurons or agglomerations of these (Gerard, 1941). The brain's activity seems, therefore, to be the result of the interplay of the central autonomous forces and the excitations coming from the peripheral organs. When a particular peripheral sense organ (e.g., eye) is stimulated, the electrical reaction is primarily in the afferent pathway (optic nerve, tract, radiation in the case of the eye) and in the particular cortical field of projection (area striata).

THE DEVELOPMENT OF THE NEURONS AND OF THE NERVOUS TISSUE

The mature neurons of the entire nervous system are derived from the embryonic ectoderm; an exception is the peripheral olfactory neurons which develop from the specific sensory epithelium of the nasal sacs. Likewise of ectodermal origin are the neuroglial cells (with the probable exception of microglia), the neurolemmal cells of the peripheral nerves and the satellite cells of the peripheral ganglia—and apparently also, certain elements of the meninges

In early embryonic stages the future central nervous system is separated by folding from the primitive ectoderm to form the *neural tube*. Next, other cells are detached from the neural tube to form internal cellular bands between the neural tube and the ectoderm that later becomes epidermis These bands are the *neural crests* that soon become segmented, the precursors of the cranial and spinal ganglia. The primitive epithelium that forms the walls of the neural tube is gradually differentiated into spongioblasts, which will become the ependyma and neuroglia, and neuroblasts, the future neurons of the brain and spinal cord. In a similar way some cells of the neural crests become peripheral neurons; others, satellite cells; still others, Schwann's neurolemmal cells. These ectodermal tissues are at first sharply separated from the surrounding mesodermal mesenchyme, from which the meninges and the connective tissue of the central nervous system and of the ganglia are derived. The intimate association of the ectodermal nervous and the mesodermal connective tissue found in the adult is achieved only gradually.

The sensory neurons of the craniospinal nerves arise from the cells which remain in the vicinity of the original neural crests where they form ganglia of these nerves. The peripheral (dendritic) processes of these cells grow outward and are modified into the axis cylinders of the sensory nerve fibers. Their central processes, the axons proper, enter the central nervous system as dorsal or sensory roots and terminate with teledendrons. The cell bodies of the peripheral motor and visceral neurons remain within the brain or spinal cord, their axis cylinders forming the ventral or motor roots of the peripheral nerves and terminating in the muscles or in the visceral ganglia. Some of the indifferent cells leave the territory of the central nervous system and migrate into various parts of the body; these become sympathetic or visceral ganglia. The steps by which the various cells of the central nervous system are derived from the primitive undifferentiated epithelium of the neural tube are illustrated in Figs. 195, 196, 197.

As soon as the immature neurons of the neural tube and crest can be distinguished from other cells they are called *neuroblasts* (Fig. 198). Those of the spinal ganglia (*o*) send their axons through the dorsal roots (*B*) into the spinal cord; those in the ventral part of the neural tube (*e*) send their axons through the ventral roots (*A*) outward toward the muscles and viscera; those in the dorsal part of the neural tube (*a*) become correlation neurons of the spinal cord. The protoplasm of the growing axons shows ameboid movements and insinuates itself between the other tissue elements by a positive outgrowth. At its advancing tip there is a bulbous enlargement called "growth cone" (Fig. 200) from which slender, spinelike projec-

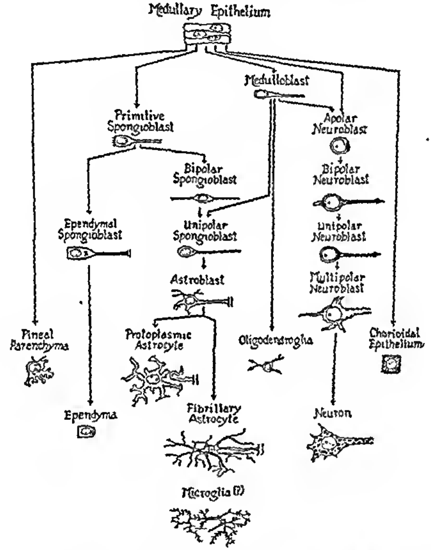

Fig. 195. Diagram, partly hypothetical, of the histogenesis of the central nervous system. Especially hypothetical are the medulloblasts; yet their existence seems to be necessary to explain the histogenesis of the nervous system, its malformations, and its tumors. After Bailey.

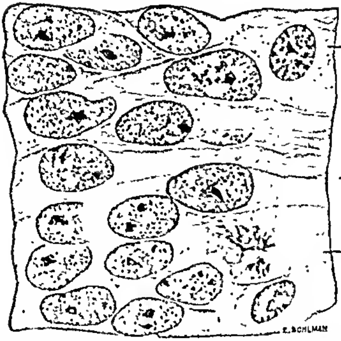

Fig. 196. A portion of a neural fold of an 18 somite human embryo (about twenty-four days old). The section was taken just caudal to the posterior neuropore. It is a pseudostratified epithelium with the cell boundaries clearly defined. The internal limiting membrane is developing but the external has not yet appeared. As in most rapidly growing tissues, there is an occasional degenerating cell. Embryo H1516 Univ. of Chicago Emb. Coll. Courtesy of G. W. Bartelmez. 1520 ×.

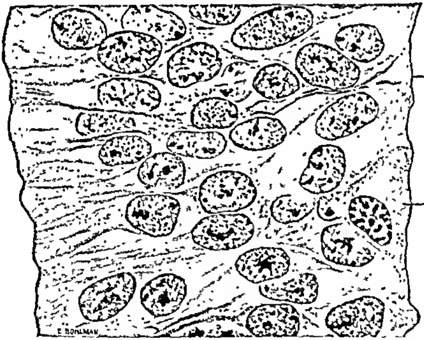

Fig. 197. A part of the lateral wall of the neural tube in the region of the medulla oblongata from a 26 somite human embryo (about four weeks old). The cells are much longer and more irregular than in Fig. 196, but it is occasionally possible to follow one through the entire thickness of the wall and the presence of cell boundaries indicates that the original epithelial condition has persisted. Both external and internal limiting membranes are present. Embryo H1382 Univ. of Chicago Emb. Coll. Courtesy of G. W. Bartelmez. 1520 ×.

tions are thrust between obstructing cells and fibers (a few such are seen in Fig. 198, *d, h, i*).

The knowledge of the development of the neurons expounded above is based chiefly upon the study of the fixed and stained material by His, Ramón y Cajal, Lenhossék, Neal, *et al.*, and was

in all essential points confirmed by Harrison's observations on the living nervous tissue and his brilliant experiments on the developing frog. This was amplified by studies of the growing nerves in the transparent tail of the living frog tadpole by Speidel. These observations showed that the

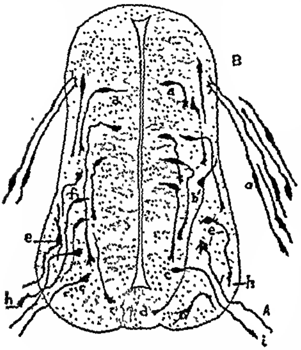

Fig. 198. Section through the spinal cord of a three-day chick: *A*, Anterior and, *B*, posterior roots; *a*, *b*, *c*, etc., neuroblasts whose axons frequently end in swellings (*d*, *h*, *i*); *o*, spinal ganglion cells whose processes have penetrated the spinal cord. *Golgi method. Redrawn* after Ramón y Cajal.

axons of the unipolar neuroblasts grow into the intercellular spaces as slender, naked protoplasmic strands (Figs. 199, 200). Nor do they form a true syncytium, but each pursues its separate course (secondary anastomoses between peripheral naked axons are the rule, however, according to Speidel).

From the observations of Speidel it is clear that in the peripheral nerve fibers all newly-formed nerve sprouts are at first completely devoid of either the neurolemmal or myelin sheaths Next they are joined by Schwann's cells but remain unmyelinated. The earliest myelin appears near the nucleus of the sheath cells from which locality it spreads proximally and distally. This demonstrates the essential rôle of the sheath cells in the process of myelinization, the details of which are still unknown. The myelinization, as a rule, begins proximally, close to the body of the neuron, and gradually progresses toward the distal end of the axis cylinder

The forces that in the course of phylogeny and ontogeny have brought about the differentiation of the neuroblasts and created the complex nervous tissue of the adult vertebrate are incompletely known. W. His and, later, in a sense, Harrison saw in *mechanical factors* the chief cause directing the growth of the neuroblasts and their expansions. Ramón y Cajal thought that in addition the various peripheral tissues secrete certain substances which exert *chemotactic influences* upon the sensitive growing buds of the axons; this process he termed *neurotropism*. He believed that in the central nervous system a similar force attracts the dendrites and the bodies of the neurons toward the points whence this force emanates.

A not unlike conception, pronounced by Strasser, was later elaborated by Ariëns Kappers under the name *neurobiotaxis*. This theory assumes the driving force to be the difference in the electric potential between the dendrites and the axis cylinder of a neuron. As a result, a neuron, or a group of them, is attracted by the axis cylinders and endings of other related neurons, this attraction being at first expressed by the shortening of the dendrites and later by the migration of the bodies in the direction of the source whence their stimuli come (that is, toward the negative potential). Child has combined

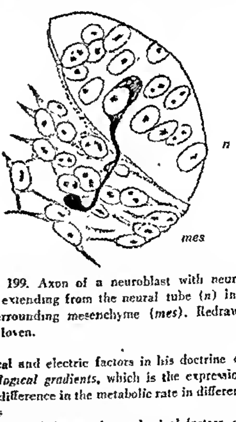

Fig. 199. Axon of a neuroblast with neurofibrils extending from the neural tube (*n*) into the surrounding mesenchyme (*mes*). Redrawn after Hoven.

chemical and electric factors in his doctrine of *physiological gradients*, which is the expression of the difference in the metabolic rate in different regions

The rôle of the *purely mechanical factors*, of the oriented ultra-structure (micellar orientation and aggregation), as the guide along whose

channels the developing axis cylinders spread, has lately been experimentally tested and the importance of the chemotactic, electrical and electromagnetic factors questioned by Weiss (1934). Speidel, too, not seeing any effect of the galvanic or faradic current upon either the rate or the direction of the growth of nerves in the living vertebrate, likewise favors the structural-stereotropic agencies, in addition to the chemical, electrical, and the radiative factors.

In any case, the question must be raised as to the ultimate cause which on the one hand determines the orientation of the ultramicroscopic micellar units in the media wherein the nervous processes expand, and on the other hand induces the selecting of particular micellar pathways by particular axons. The factors determining the growth and the synaptic interconnections of the neurons and the evolution and development of the nervous system in general may be many

Fig. 200 Isolated unipolar neuroblast with a forked process, from a part of the neural tube of *Rana palustris* explanted in a drop of lymph of *Rana pipiens* two days previously. 350 ×. Redrawn after Harrison.

and complex, and delicately balanced in quality, quantity, space, and time. Chemical, electrical and other possible, but as yet undisclosed, agencies may first influence the micellar orientation which in turn may determine the direction of growth and the establishment of relationships of neurons. But the chief driving force is unknown

The nerve cells, on the contrary, as soon as they reach that very early stage of differentiation when they can be recognized as neuroblasts, appear to lose the power of multiplication. If any neurons are destroyed, they are not replaced. Moreover, most of the neurons of the adult body have their own individual connections and functions. No two of them are exactly alike. It is true that neurons of similar structure and with similar connections are often associated as a "system" in the performance of some particular nervous function, and if some of these neurons are destroyed, the remaining members of the system may continue to perform the function in question. But if the entire system is destroyed, this function is irreparably lost.

Though mature neurons cannot proliferate, they show visible changes in the course of normal physiological activity and in various pathological conditions. The change in size and shape is particularly manifest in the following three instances: (1) transplantation or explantation of nerve cells, (2) pathological or operative destruction of portions of the central nervous system, and (3) after injury to peripheral nerves.

In the nervous system of both vertebrates and invertebrates, even in nerve cells of the same variety, the internal structure is not quite identical. The difference depends in part on the changing functional conditions of the individual cells for, at the moment of fixation some cells were active, while others were resting. By experimentally stimulating the nervous elements it is possible to carry such changes to an extreme degree. On the other hand, by investigating the nervous elements of animals during hibernation, one can obtain an idea of the structure of neurons during almost complete rest.

The chromophil substance of Nissl is especially sensitive to both artificial stimulation and normal fatigue. In nervous elements which were highly

DEGENERATION AND REGENERATION OF THE NERVOUS TISSUE

The neuroglia of the adult central nervous system and the cells of Schwann and allied elements of the peripheral system are far less specialized in structure and function than are the neurons. Under certain circumstances they are capable of returning to an embryonic condition, and of rapid and apparently indefinite proliferation. This occurs in certain tumors (gliomas), in scar tissue, in various other pathological conditions, and in a very special form during the process of regeneration of peripheral nerve fibers.

active or were in some way impaired the chromophil substance partly disintegrates into granules distributed throughout the cytoplasm and partly dissolves. This phenomenon is called *chromatolysis* or *tigrolysis* (Fig. 201). The Nissl substance may even disappear completely in consequence of extreme fatigue or exhaustion. After a period of rest this substance again accumulates cytoplasm becomes vacuolated, and finally the cell perishes. During intense activity the neurofibrils likewise increase in number and become thinner and paler. During hibernation they diminish in number, become thicker and more deeply stained.

The comportment of chromophil substance in fatigue and in various pathological conditions

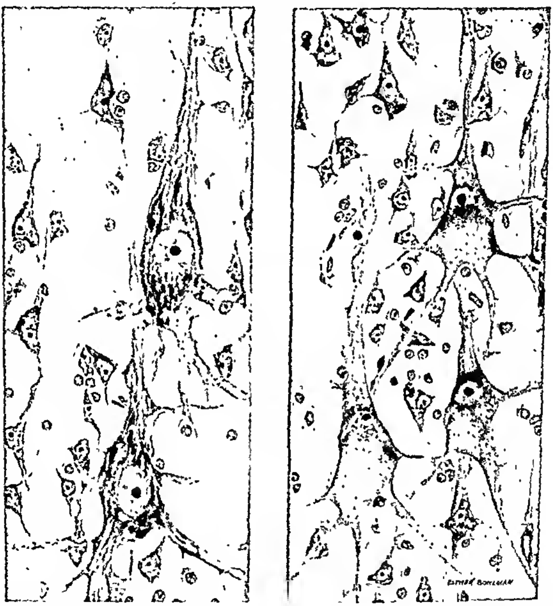

Fig 201. On the left side are normal cells of the motor cortex of a macaque; the right side shows chromatolysis in similar cells after hemisection of the cervical spinal cord Stained after Nissl-Lenhossék. Courtesy of S. Polyak.

and the cell assumes its original appearance. If too high a degree of fatigue is produced, complete degeneration and death of the neuron may result.

Accompanying chromatolysis there may be an increase of the volume of the cell due to an increased water content of both nucleus and cytoplasm. With the advance of exhaustion of the cell, a decrease in its volume is evident; the has been much studied. Most of these changes take the form of chromatolysis, with considerable variety in detail. The most complete observations concern the change in the cell body after its axon is severed. This is the so-called *primary reaction of Nissl*, the axon reaction or the *retrograde cell degeneration*. The cell body grows larger, chromatolysis is observed, the nucleus

2. Axon Reaction.
3. Retrograde cell degeneration.
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migrates to one side of the body and the nuclear membrane shrivels. The reaction may be more or less severe depending on the variety of the neuron, the nature of the injury, the distance from the cell body at which the axon was cut, and the degree of regeneration, if any, which follows the injury. Where the axon is not destroyed completely, so that the remaining part may still perform some function, and where regeneration occurs, the injured neuron may make a complete recovery. In more severe injury the changes in the cell body are more rapid and continue until degeneration is complete and the cell dies. In the brain the neurons probably always degenerate and disappear completely whenever their axis destroyed, degenerate, and during the period of from one to twelve weeks or more after the injury, before the degenerating myelin is resorbed it can be specifically stained by a *method devised by Marchi*. Since the altered myelin stains black in contrast to the unstained myelin

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retrograde chromatolysis and the method of Marchi—are extensively used in the investigation of the fiber tracts of the brain and the spinal cord.

When a nerve trunk is severed the peripheral or distal portion soon loses its glossy white aspect and becomes dull and gray. The central

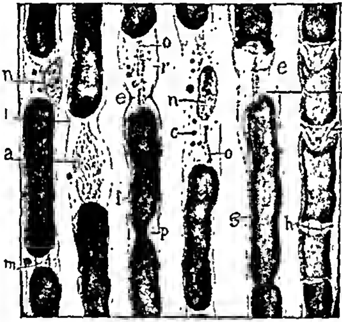

Fig 202. Fibers of the peripheral stump of a rabbit killed twenty-four hours after section of the nerve. *a*, Fusiform widening of the axon next to a constriction (*i*); *b*, plastic vacuole of the constrictions, *c*, spherules of Erholz; *e*, cementing disk; *f*, normal incisure of Schmidt-Lantermann; *g*, Infundibulum of the incisures; *h*, fissural rings; *m*, protoplasmic bridges being formed at the level of the incisures; *n*, nuclei; *o*, subsistent axon in the protoplasm of Schwann's cells; *r*, protoplasm accumulated next to the disks of Ranvier. After Ramón y Cajal.

cylinders are interrupted. The axis cylinder likewise exhibits immediate changes in form and structure after subjection to drugs, *x*-rays, heat, cold, starvation, and after mechanical injury.

It is difficult and frequently impossible in normal material to follow the axons of some particular group of nerve cells as they make their devious ways through the central or peripheral nervous system, mingled with other fibers. If some of these fibers are severed by accident or disease, or if they are cut in an experiment, the cell bodies from which the injured fibers arise will show chromatolysis. On the other hand, the myelinated fibers which have been separated from their cell bodies, or whose cells have been or proximal portion remaining continuous with the cell bodies apparently does not change much. Artificial stimulation of the peripheral portion three or four days after the operation fails to produce a contraction of the muscles which it supplies, if it contains motor fibers. However, stimulation of the central portion, if it be a sensory or mixed nerve, produces the usual pain sensations and more or less widespread motor reflexes. Microscopic examination shows that the immediate result of the operation is a primary degenerative change which involves the ends of both the central and peripheral portions for a short distance This is, *traumatic degeneration*. But the changes in the whole length of the

peripheral portion depend upon a different process. This is called *secondary or Wallerian degeneration* of the nerve fiber (Fig. 202). It affects the entire length of the peripheral portion of the nerve, including its terminal apparatus.

In Wallerian degeneration those parts of the severed peripheral fiber which are organic parts of the neuron, and hence trophically dependent upon its cell body, undergo complete degeneration. However, if conditions are favorable for regeneration, they may be restored to perfect function. This applies to the axis cylinder and to the myelin sheath if present. On the other hand, the sheath of Schwann being trophically independent of the neuron, survives and is reorganized with the active proliferation of nuclei and the growth of cytoplasm. It assists in the regeneration of the axon (see below). It is possible to induce degeneration of peripheral axons by vitamin B deficiency without seriously altering the myelin sheath (E. Clark).

The axis cylinder of the peripheral stump always perishes. But in many cases there is ultimately a complete *regeneration* of this degenerated portion of the nerve and its terminal apparatus (Fig. 203). This regenerative process always proceeds from the end of the central stump. It progresses much slower than the rapidly occurring Wallerian degeneration. It is accomplished relatively easily and quickly when the two severed ends of the nerve are immediately brought into close contact with each other. If the ends have been widely separated the regenerative process may require a long time or never take place. It occurs much more rapidly and successfully in a young individual than in an old one.

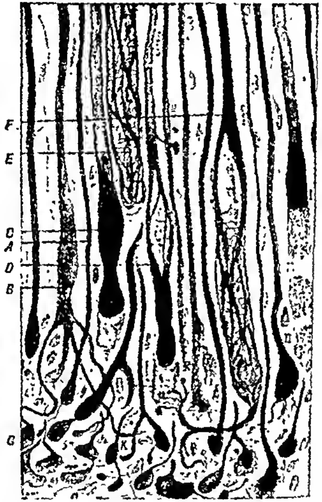

Fig. 203 Central stump of the sciatic nerve of an adult cat, killed two days after the section: *A*, Nonmedullated fiber; *B*, medullated axon ending in terminal branches; *E*, *F*, structures of Perroncito. After Ramon y Cajal.

The nonmyelinated fibers also undergo a secondary degeneration peripheral to the injury. Here, too, the axis cylinder disintegrates and its fragments are absorbed by the protoplasm of Schwann's sheath. This process differs from that in the myelinated fibers only by the absence of the myelin sheath.

The degenerative processes cannot be sharply

separated from those of regeneration, for the entire response of the neuron to injury seems to be reparative from the start. It is now definitely established that the regeneration can be carried through to completion only by the outgrowth of nerve fibers from the uninjured axons of the central stump. Bungner's strands of Schwann's cells do not develop axons of their own, but are penetrated by new axons growing from the central stump of the nerve.

CONNECTIVE TISSUE, CHORIOID PLEXUS, VENTRICLES, AND MENINGES OF THE CENTRAL NERVOUS SYSTEM

In addition to the neurons and the supporting neuroglia, both of ectodermal origin, the brain and the spinal cord everywhere contain blood vessels derived from mesenchyme. The membranes enveloping the brain and spinal cord are likewise composed chiefly of connective tissue. There are three such membranes. The outermost, the dura mater or pachymeninx, is dense and firm. Both the inner membranes, the innermost or the pia mater and the one next to it, the arachnoid membrane, are composed of much looser connective tissue and are called leptomeninges. The dura and arachnoid membranes are separated by the subdural space; the space between the outer layer of arachnoid and pia is called subarachnoid. Both spaces contain very loosely arranged connective tissue and are filled with cerebrospinal fluid.

Dura Mater. The relation of the dura to the surrounding bones differs in the spinal cord and in the brain. In the vertebral canal the inner surface is lined by its own periosteum, and within this a separate cylindrical dural membrane loosely encloses the cord. There is a rather wide epidural space between the periosteum and the dura which contains much loose connective and fatty tissue with many veins. The dura is firmly connected to the spinal cord on each side by a series of denticulate ligaments. The inner surface of the spinal dura is lined with squamous cells. Its collagenous bundles run for the most part longitudinally, and the elastic nets are less prominent than in the cerebral dura.

The dura mater of the brain at the beginning of its embryonic development also has two layers, but in the adult these are more or less closely joined. Both consist of loose connective tissue with elongated fibroblasts. The outer layer adheres to the skull rather loosely except at the sutures and the base of the skull. It serves as periosteum, is looser and richer in cells than the inner layer, and contains many blood vessels; its thick collagenous fibers are arranged in bundles. The inner layer is thinner with finer fibers forming an almost continuous sheet. Its fibers run from in front and below backward and upward, thus with orientation opposite to those of the outer layer. These fibers are so arranged as to equalize tensions and pressures within the cranial cavity. The inner surface of the dura is smooth and covered with a layer of squamous mesothelial cells.

Arachnoid. In the brain and spinal cord the leptomeninges are similar in structure. The arachnoid is a thin, netlike membrane devoid of blood vessels, resembling the transparent parts of the omentum. Its outer surface is smooth, but from its inner surface run a multitude of thin, branching threads and ribbon-like strands, attached to the pia. The tissue on macroscopic examination has a cobweb-like appearance. The arachnoid membrane bridges over the sulci and the fissures on the surface of the brain and the spinal cord, forming subarachnoid spaces of various extent within these sulci.

Pia Mater. This inner membrane is a thin connective tissue net that closely adheres to the surface of the brain and the spinal cord. It contains a large number of blood vessels from which most of the blood of the underlying nervous tissue is derived. Attached to the pia are the inner fibrous strands of the arachnoid and these

peripheral portion depend upon a different process. This is called *secondary or Wallerian degeneration* of the nerve fiber (Fig. 202). It affects the entire length of the peripheral portion of the nerve, including its terminal apparatus.

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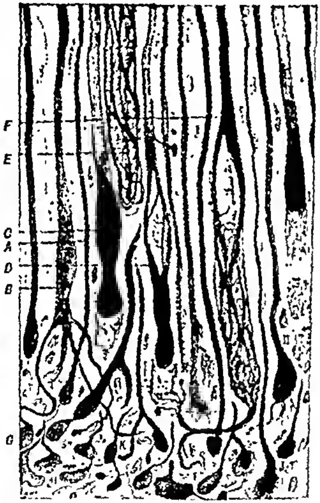

Fig. 203. Central stump of the sciatic nerve of an adult cat, killed two days after the section: *A*, Nonmedullated fiber, *B*, medullated axon ending in terminal branches; *E*, *F*, structures of Perroncito. After Ramón y Cajal.

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The degenerative processes cannot be sharply

cord. Afferent nerve endings are also present, but are very unevenly distributed.

Both myelinated and unmyelinated nerve fibers accompany the blood vessels into the substance of the spinal cord and the brain, ending on the muscle cells of the vessels. These come from similar nerves of the pial vessels, and the two nervous plexuses are continuous.

Chorioid Plexus. There are four places where the wall of the brain retains its embryonic character as a thin, nonnervous epithelium. This part of the brain wall is the lamina epithelialis. The pia mater which covers it is extremely vascular and otherwise modified to form a chorioid plexus. The lamina epithelialis is very closely joined to the chorioid plexus and the whole is called tela chorioidea, or less exactly, chorioid plexus.

These chorioid plexuses are found in the roof of the third and fourth ventricle, and in a part of the wall of the two lateral ventricles. In each case the tela chorioidea is much folded and invaginated into the ventricle, so that the free surface exposed to the ventricular fluid is very large, with branching tufts of tortuous vessels and a rich capillary net.

The epithelium early acquires a peculiar structure, different from that of the ependymal cells lining the ventricles. In embryonic stages it contains glycogen and carries cilia. In the adult its cells are cuboidal and are arranged in a single, regular layer. Each contains a large, round nucleus and a varying number of rod-shaped and granular mitochondria. Very common inclusions are large, transparent vacuoles in the distal part of the cell, or large, usually single, fat droplets On the free surface some have a brushlike border and, in the guinea pig, long, motile cilia. In animals repeatedly injected intravenously with vital dyes, such as trypan blue, the epithelium of the chorioid plexus stores large amounts of the dye in granular form. In the perivascular connective tissue core of the plexus are many fixed macrophages which store large amounts of dye in contrast to those of the leptomeninges

Blood Vessels of the Central Nervous System. The arteries reach the spinal cord with the ventral and dorsal nerve roots (anterior and posterior radicular arteries) and form a dense arterial network in the spinal pia mater. Here several longitudinal arterial pathways can be distinguished (spinal arterial tracts). The most important among them is the anterior arterial tract; it gives off a multitude of small branches (central arteries) which enter the ventral medial fissure and penetrate to the right and left into the medial part of the anterior gray columns. They supply the major part of the gray substance with blood. Numerous smaller branches of the pial arterial net, the peripheral arteries, penetrate the white substance of the cord along its entire circumference. The capillary nets in the white substance are loose and have meshes which are drawn out longitudinally. The capillaries of the gray substance are much more numerous and dense. The course of the veins does not correspond with that of the arteries. Numerous venous branches emerge from the periphery of the cord and from the ventral median fissure and from a diffuse plexus in the pia; this is especially prominent on the dorsal surface of the cord. From this plexus the blood is led away by veins accompanying the ventral and dorsal roots.

The arterial supply of the brain is derived almost entirely from the carotids and the large arteries at its base, chiefly the basilar artery and the circle of Willis. Most of the arteries from these large vessels pass upward in the pia mater, from which smaller vessels dip into the brain substance. These vessels after penetrating the brain were commonly supposed to be end arteries, with no appreciable amount of anastomosis from one to another; this problem requires further study in mammals.

As in the spinal cord, the capillary net in the cerebral white matter is relatively meager, with elongated meshes; in the gray matter the net has a closer mesh. It is assumed that the density of capillaries is a crude indication of the rate of metabolism of the tissue supplied by it. On this assumption it is clear that the metabolism of the gray substance is much more active than that of the white.

The linear extent of capillaries per unit volume of brain substance has been measured by Craigie in a number of representative parts of the central nervous substance in various animals. He finds, for instance, in the rat that parts of both white and gray matter differ in vascularity, all the gray being more vascular than the white. The motor nuclei are less vascular than the sensory nuclei and correlation centers. In the cerebral cortex the fourth layer of Brodmann is more vascular than the other layers, and the supragranular layers tend to be more vascular than the infragranular layers. The parietal area is more vascular than the others, and the vascularity of the cerebellar cortex is about the same as that of the cerebral cortex taken as a whole. In studying the postnatal development of

two membranes are so intimately related that their histological structure can best be described together. In fact, these two membranes are often treated as one, the *pia-arachnoid.*

The main elements of both the arachnoid and the pia are interlacing collagenous bundles surrounded by fine elastic networks. In the spinal pia an outer longitudinal and an inner circular layer can be distinguished. Among the cells are fibroblasts and fixed macrophages; these are especially numerous in the pia along the blood vessels. They correspond in their general histological properties to the macrophages of the other parts of the body. They store vital dyes injected directly in the subarachnoid space. In inflammation, especially in tuberculous meningitis, they are transformed into large free macrophages or epithelioid cells. In man they often contain, even under apparently physiological conditions, considerable amounts of a yellow pigment that sometimes reacts positively to tests for iron. When vital dyes are injected intravenously into a living animal, the macrophages of the leptomeninges store only very small quantities of them; at the same time, the tissues of the central nervous system (except the chorioid plexus) remain practically colorless, at least in adult animals. Thus, the walls of the blood vessels of the leptomeninges seem to be an unsurmountable barrier for some of the vital dyes that have entered the general circulation. In young animals given intravenous dye injections, however, there can be found a distinct, although small storage of the dye in the nerve cells in different places of the brain stem, so that the apparent impermeability of the walls of the blood vessels develops gradually.

Along the blood vessels of the pia mater are scattered single mast cells and small groups of lymphocytes. In certain pathological conditions the latter increase enormously in numbers and may become transformed into plasma cells. The tissue of the leptomeninges, especially along the blood vessels of the pia, also contains many embryonic mesenchymal elements. In the pia mater, particularly on the ventral surface of the medulla oblongata, a varying number of melanoblasts can be found.

The outer and the inner surfaces of the arachnoid, the trabeculae, and the outer surface of the pia are lined with a layer of squamous mesenchymal epithelial cells. Whereas some investigators describe their rounding off, mobilization, and transformation into free macrophages under the influence of inflammatory stimuli, others trace the origin of macrophages exclusively to fixed macrophages. This question requires further study.

During development of the meninges two zones may be distinguished: an outer zone of condensation of mesenchyme which gives rise to periosteum, dura, and membranous arachnoid, and an inner zone which becomes pia. Between these two zones the mesenchyme remains loose and later forms spongy tissue permeating the subarachnoid spaces.

In lower vertebrates the mesenchyme of the head is formed of cells derived in part from the entoderm (mesentoderm) and in part from the ectodermal neural crest (mesectoderm). Both types of mesenchyme have been shown to participate in the formation of the meninges. Working with amphibians and birds, Burr and others have transplanted portions of the early neural tube with and without the neural crest into foreign tissue. In subsequent development the transplants with the neural crest acquire typical pia mater containing cells of neural crest origin, while those lacking the neural crest show an atypical and defective pia.

If the mammalian pia mater likewise contains elements derived from both mesectoderm and mesentoderm, then it remains uncertain whether the microglia, which is said to migrate into the brain from the pia mater, is ultimately of ectodermal or entodermal origin. It has been suggested, moreover, that this twofold origin of the elements of the leptomeninges explains some peculiarities of meningeal tumors.

Nerves of the Meninges. The dura and pia are richly supplied with nerves. All vessels of the pia and of the chorioid plexus are surrounded by extensive nervous plexuses in the adventitia, from which very fine fibrils penetrate the media. These nerves have their origin in the carotid and vertebral plexuses and in certain cranial nerves, and belong to the sympathetic system. Some fibers seem to emerge directly from various places of the brain. Sensory, nonencapsulated, nerve terminations, and even single nerve cells, are also present on the adventitia of the blood vessels.

The cerebral dura contains, besides the nerves of the vessels, numerous sensory nerve endings in its connective tissue. The connective tissue of the cerebral pia contains extensive nervous plexuses. They are especially abundant in the tela chorioidea of the third ventricle. The fibers end either in large, pear-shaped, or bulbous swellings or in skeins and convolutions similar to those of the corpuscles of Meissner. In the spinal pia the vessels receive their nerves from the plexuses following the larger blood vessels to the

cord. Afferent nerve endings are also present, but are very unevenly distributed.

Both myelinated and unmyelinated nerve fibers accompany the blood vessels into the substance of the spinal cord and the brain, ending on the muscle cells of the vessels. These come from similar nerves of the pial vessels, and the two nervous plexuses are continuous.

Chorioid Plexus. There are four places where the wall of the brain retains its embryonic character as a thin, non-nervous epithelium. This part of the brain wall is the lamina epithelialis. The pia mater which covers it is extremely vascular and otherwise modified to form a chorioid plexus. The lamina epithelialis is very closely joined to the chorioid plexus and the whole is called tela chorioidea, or less exactly, chorioid plexus.

These chorioid plexuses are found in the roof of the third and fourth ventricle, and in a part of the wall of the two lateral ventricles. In each case the tela chorioidea is much folded and invaginated into the ventricle, so that the free surface exposed to the ventricular fluid is very large, with branching tufts of tortuous vessels and a rich capillary net.

The epithelium early acquires a peculiar structure, different from that of the ependymal cells lining the ventricles. In embryonic stages it contains glycogen and carries cilia. In the adult its cells are cuboidal and are arranged in a single, regular layer. Each contains a large, round nucleus and a varying number of rod-shaped and granular mitochondria. Very common inclusions are large, transparent vacuoles in the distal part of the cell, or large, usually single, fat droplets. On the free surface some have a brushlike border and, in the guinea pig, long, motile cilia. In animals repeatedly injected intravenously with vital dyes, such as trypan blue, the epithelium of the chorioid plexus stores large amounts of the dye in granular form. In the perivascular connective tissue core of the plexus are many fixed macrophages which store large amounts of dye in contrast to those of the leptomeninges.

Blood Vessels of the Central Nervous System. The arteries reach the spinal cord with the ventral and dorsal nerve roots (anterior and posterior radicular arteries) and form a dense arterial network in the spinal pia mater. Here several longitudinal arterial pathways can be distinguished (spinal arterial tracts). The most important among them is the anterior arterial tract; it gives off a multitude of small branches (central arteries) which enter the ventral medial fissure and penetrate to the right and left into the medial part of the anterior gray columns. They supply the major part of the gray substance with blood. Numerous smaller branches of the pial arterial net, the peripheral arteries, penetrate the white substance of the cord along its entire circumference. The capillary nets in the white substance are loose and have meshes which are drawn out longitudinally. The capillaries of the gray substance are much more numerous and dense. The course of the veins does not correspond with that of the arteries. Numerous venous branches emerge from the periphery of the cord and from the ventral median fissure and from a diffuse plexus in the pia; this is especially prominent on the dorsal surface of the cord. From this plexus the blood is led away by veins accompanying the ventral and dorsal roots.

The arterial supply of the brain is derived almost entirely from the carotids and the large arteries at its base, chiefly the basilar artery and the circle of Willis. Most of the arteries from these large vessels pass upward in the pia mater, from which smaller vessels dip into the brain substance. These vessels after penetrating the brain were commonly supposed to be end arteries, with no appreciable amount of anastomosis from one to another; this problem requires further study in mammals.

As in the spinal cord, the capillary net in the cerebral white matter is relatively meager, with elongated meshes; in the gray matter the net has a closer mesh. It is assumed that the density of capillaries is a crude indication of the rate of metabolism of the tissue supplied by it. On this assumption it is clear that the metabolism of the gray substance is much more active than that of the white.

The linear extent of capillaries per unit volume of brain substance has been measured by Craigie in a number of representative parts of the central nervous substance in various animals. He finds, for instance, in the rat that parts of both white and gray matter differ in vascularity, all the gray being more vascular than the white. The motor nuclei are less vascular than the sensory nuclei and correlation centers. In the cerebral cortex the fourth layer of Brodmann is more vascular than the other layers, and the supragranular layers tend to be more vascular than the infragranular layers. The parietal area is more vascular than the others, and the vascularity of the cerebellar cortex is about the same as that of the cerebral cortex taken as a whole. In studying the postnatal development of

this vascular pattern, it was concluded that the richness of the capillary supply is directly related to functional activity, and that the metabolism involved in the latter requires a greater blood supply than does the metabolism of growth.

There are no lymphatics in the central nervous system. Blood fluids which pass out from the capillaries seep through the tissue and are not collected in lymphatic vessels, as in most other parts of the body. The blood vessels that penetrate from the pia mater are surrounded by perivascular spaces which open freely at the brain surface into the subarachnoid spaces. Thus the cerebrospinal fluid, *derived from the blood*, is drained from the brain tissue outward toward the meninges without at any time being enclosed in definite lymphatic vessels.

Ventricles. The central nervous system begins its development as a neural tube with a wide cavity throughout its length, and it preserves its character as a hollow organ throughout life. The ventricle of the spinal cord, or central canal, in the adult is very minute, or it may be obliterated. It does not seem to perform any important function. But in the normal adult the ventricular cavities of the brain always form a continuous channel for flow of cerebrospinal fluid throughout its length. If any part of this channel is occluded by disease so as to prevent free circulation of its fluids, an increased intracerebral pressure develops, with resulting hydrocephalus or other serious pathological consequences.

The ventricular cavity is dilated in four regions: the two lateral ventricles in the cerebral hemispheres, the third ventricle in the thalamic region, and the fourth ventricle in the medulla oblongata and pons. Chorioid plexuses develop in these four regions, and most of the ventricular fluid is derived from the blood vessels of these plexuses.

Meningeal Spaces. Between the dura mater and the arachnoid, the subdural space is comparable to a serous cavity. It contains a minimum of fluid and in reality is scarcely more than a potential space. Between the outer sheet of arachnoid and the pia, the *subarachnoid space* is traversed by cobwebby connective tissue trabeculae. It is independent of the subdural space and contains a large amount of fluid. At the summits of the convolutions it is narrow, but in the sulci it is wide and deep. The subarachnoid space is especially wide throughout the length of the spinal cord. In the brain it is greatly enlarged in a few places termed "cisterns" where the arachnoid is widely separated from the pia and the trabeculae are rare or absent. The most important of the cisterns lies above the medulla oblongata and below the posterior border of the cerebellum (cisterna cerebellomedullaris, or cisterna magna). The fourth ventricle communicates with this cistern through three openings in the tela chorioidea, a medial foramen of Magendie—recently questioned by Meulen—and the two lateral foramina of Luschka.

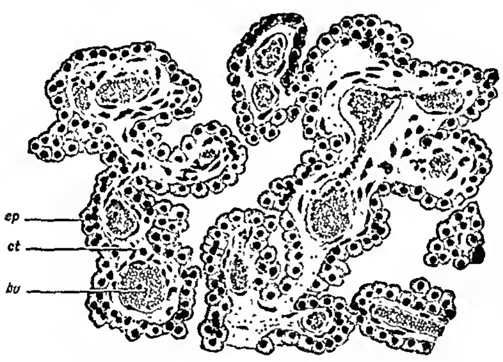

Fig. 204. Chorioid plexus of the fourth ventricle from man: *ep*, Epithelium; *ct*, connective tissue; *bv*, blood vessels. 190 ×.

Cerebrospinal Fluid. The central nervous system is surrounded on all sides by cerebrospinal fluid; it is suspended in it as in a water-bed. This fluid protects it from concussions and mechanical injuries and is of importance for its metabolism. The subarachnoid spaces are in free communication so that cerebrospinal fluid may pass through them from end to end of the central nervous system. The amount of the fluid is variable, estimated as 80 to 100 cc., or even as much as 150 cc. It is limpid, slightly viscous and has a low specific gravity (1.004–1.008). It contains traces of proteins, small quantities of inorganic salt and dextrose, and very few lymphocytes (about 2 or 3 and not more than 10 in 1 cu. mm.). It resembles the aqueous humor of the eye more closely than any other liquid of the body.

The cerebrospinal fluid is constantly renewed. It circulates slowly through the brain ventricles and through the meshes of the subarachnoid spaces. If these spaces are opened to the outside by injury (subarachnoid fistula), large amounts of fluid steadily drain off—200 cc. or more in a day. The sources of this fluid are primarily the blood vessels of the chorioid plexus, the pia mater, and the brain substance. From the brain substance the flow is outward into the subarachnoid spaces; from the chorioid plexus it is inward into the ventricles. Fluid may be added to the ventricles in a few other places, notably in the area postrema at the lower end of the fourth ventricle. The ependymal surfaces in general do not seem to discharge fluid into the ventricles. On the other hand, the absorption of fluid from the ventricles into neighboring veins takes place through the ventricular walls. The plexuses are wholly secretory, not resorptive, in function. They are the chief source of the cerebrospinal fluid. The chief channel of discharge of ventricular fluid outward into the subarachnoid spaces is through specially modified localities of the membranous roof of the fourth ventricle.

The flow of ventricular fluid normally passes from the lateral ventricles of the cerebral hemispheres, where it is derived chiefly from the lateral chorioid plexuses, through the foramina of Monro into the third ventricle. Here fluid is added from the chorioid plexus and the augmented flow passes through the aqueduct of Sylvius into the fourth ventricle, where more fluid is added from the chorioid plexus. From the fourth ventricle the fluid passes into the cerebellomedullary cistern, and from here it diffuses in all directions through the subarachnoid spaces. Some of it apparently gets into the extracranial lymphatics by way of the perineural spaces within the sheaths of the cranial nerve roots, part reaching the nasal cavity along the perineural sheaths of the olfactory nerve filaments. Around the spinal nerve roots there is an arrangement of the dural veins and sinuses adapted for the passage of cerebrospinal fluid directly into the venous blood, rather than into the lymphatic vessels. A very small part of the cerebrospinal fluid enters the lymphatics or the veins by the routes just mentioned. Most of it passes directly into the big endocranial venous sinuses through the arachnoid villi.

Arachnoid Villi. The large endocranial venous sinuses are entirely enclosed by massive walls of dura mater except in definite places, chiefly in the sagittal sinus of the falx, where the dura is perforated by numerous protrusions of the arachnoid membrane, through each of which a finger-like evagination of the arachnoid mesothelium is thrust into the lumen of the sinus. This is the arachnoid villus. Its cavity, which contains a small amount of loose arachnoid tissue, is in free communication with the subarachnoid spaces, so that here the fluid of these spaces is separated from the blood of the sinus only by the very thin mesothelial membrane.

These villi have been found in dogs, cats, monkeys, human infants, and adults. In man, with advancing age, they are enlarged and in this condition have long been known as Pacchionian corpuscles (granulations).

The arachnoid villi provide the main pathway for the outflow of cerebrospinal fluid directly into the venous circulation. This flow is rapid Dyes and other chemicals injected into the subarachnoid spaces can be detected in the blood stream in from ten to thirty seconds, and only after thirty minutes can they be found in the lymphatics.

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Chapter X

THE BLOOD VASCULAR SYSTEM

MULTICELLULAR organisms require a mechanism to distribute nutritive materials, oxygen and hormones to the various parts of the body and to collect the products of metabolism from the whole body and transmit them to the excretory organs. In the vertebrates this function is carried out by the vascular system. It consists of tubelike vessels, the *arteries*, *capillaries*, and *veins*, and the central motor apparatus, the *heart*, which maintains a constant circulation of the blood by its contractions. The arteries lead from the heart to the capillaries, the veins from the latter to the heart. Lymphatic vessels are described in Chapter XI.

The circulation of the blood in a living animal may be studied directly in a thin vascular membrane, as the web of a frog's tongue, the wing of a bat, or the special chambers inserted in the ear of a rabbit (Sandison, 1932). The vessels of thicker organs may be studied with the aid of illuminated glass or quartz rods (Knisely. 1936).

CAPILLARIES

The only component of the wall of a capillary is the endothelium; this is the characteristic structure in every vessel, including the heart. In the living animal it is usually possible to distinguish the endothelial nuclei scattered along the outlines of the capillaries. After fixation and staining the wall of the capillaries stands out clearly as a thin, homogeneous membrane, within which the endothelial nuclei are located at various distances from one another.

The endothelial cells are structurally very similar to fibroblasts. The elongated or oval nucleus is flattened, sometimes curved with the lumen of the vessel, and contains fine, dustlike chromatin particles similar to those in the nucleus of the fibroblast; it lacks, however, the large nucleoli; its membrane often shows longitudinal folds. These slight differences are rapidly effaced when the endothelial cells turn into fibroblasts.

The flat endothelial cells are usually stretched along the axis of the capillary and have tapering ends. In the wider capillaries they are shorter and broader. In the lung their outlines are irregularly scalloped. In capillaries of medium width, only two curved cells surround the lumen. In wider capillaries the aperture may be surrounded by a greater number of cells while in very narrow ones a single endothelial cell may form the wall of the tube.

The caliber of the capillaries in various parts of the body of a given animal varies within narrow limits and is closely related to the size of the red blood corpuscles. In man it averages about 8 μ. Patent thin *capillaries*, through which only blood plasma circulates, probably do not exist although great numbers of the capillaries are collapsed when the organ or tissue is in a resting condition. When the organs begin to function actively, these collapsed capillaries open up and blood circulates through them. In sections of tissues fixed in the usual manner, the capillaries appear narrower than in the living animal while artificially injected capillaries are

often distended beyond their normal limits.

In the majority of capillaries it is possible to show by the injection of silver nitrate that their walls consist of separate endothelial cells whose boundaries stand out as sharply stained black lines; each cell contains a single nucleus (Fig. 205). In such preparations the cell boundaries are frequently covered with angular, dark spots. They were originally thought to be openings in the walls of the capillaries between the endothelial cells, the so-called *stigmata* or *stomata;* they are now known to be artefacts. Intravascularly injected India ink particles were seen, in the living frog, to accumulate first in the cementing lines (Chambers and Zweifach).

The capillaries originate from the embryonic connective tissue. As they penetrate everywhere between the elements of various organs and tissues, they are accompanied along their entire course by connective tissue cells and layers of thin, collagenous or reticular fibers; these closely adjoin the endothelium in most places. The network of reticular fibers forms a thin membranous sheath around the capillaries and separates them from the elements of the other tissues.

Fig. 205. Capillary from the mesentery of a frog. The boundaries of the endothelial cells are stained black with silver nitrate 350 ×. Redrawn after Ranvier.

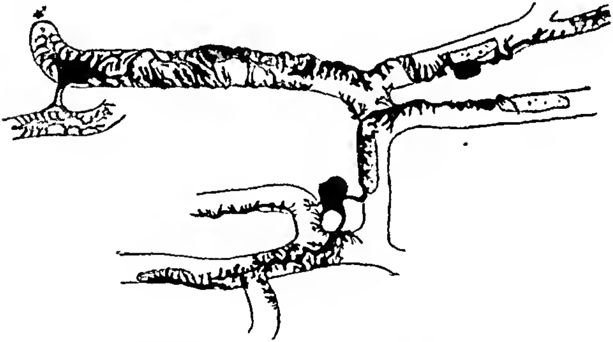

Fig. 206. Arterial capillaries from the heart of a forty-three-year old man. Four polymorphous perivascular cells continue into those of the capillaries. The arrow points toward the artery. Chrome-silver impregnation 1000 ×. Redrawn after Zimmermann

The connective tissue which accompanies the capillaries is sometimes called *perithelium.* This is an indefinite term and includes several types of cells; because of its indefiniteness, it should be discarded. The usual capillaries are ac-

companied by fixed macrophages, cells of probably undifferentiated mesenchymal nature, and a few scattered nerve cells which can only be identified through the pericapillary cells are of quite different nature. Thus, along the capillaries of the nictitating membrane of the frog's eye are peculiar cells with long, branching proc-

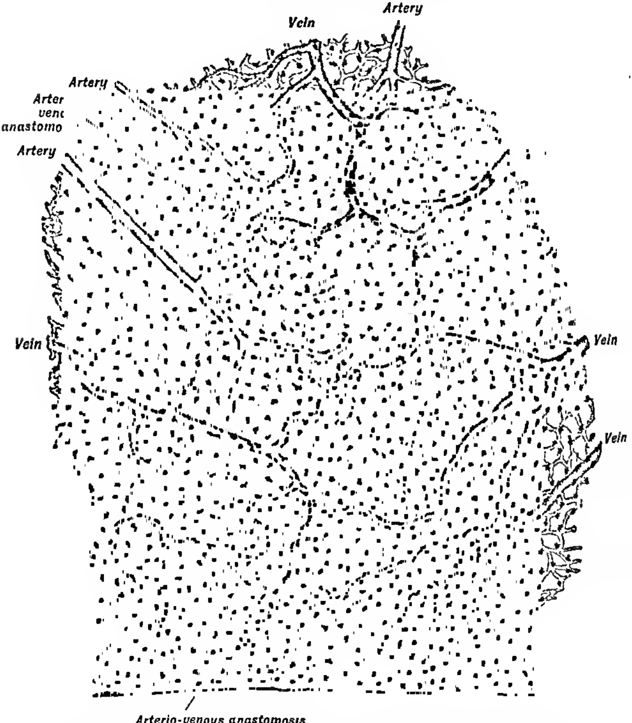

Fig. 207. Network of blood vessels in the web of a frog showing the connection of arteries and veins with the capillary network and direct connections between arterioles and venules. Many chromatophores are scattered along the capillaries. Medium magnification. (A.A.M.)

use of special histologic methods. The pericapillary mesenchymal cells are beautifully demonstrable in the serous membranes (Fig. 79). In certain instances the esses which surround the capillary wall (Rouget cells). These cells have been seen to contract under electric stimulation (Vimtrup). Hence, in this membrane,

these cells may be considered to be of the nature of smooth muscle cells. According to Zweifach the Rouget cells round up, but do not contract when prodded. Volterra claims that the Rouget cells lack the birefringent myofibrils characteristic of smooth muscle. Studies made on living blood vessels in chambers inserted in the rabbit's ear indicate that capillary contractility in the mammals does not depend on the Rouget cells (Clark). Microdissection studies have shown that the endothelial cells may contract after direct mechanical stimulation.

The fluid part of the blood reaches the elements of the tissues only by passing through the endothelial protoplasm of the capillaries. Leukocytes pass through the wall by their ameboid movement in which they push apart the processes of the endothelial cells, or pass through the protoplasm of these cells and form temporary openings which close immediately after their passage (Fig. 89).

The capillaries connect the terminal branches of arteries and the beginnings of the veins; they always form extensive networks by their frequent branchings and anastomoses; these networks thoroughly penetrate the various tissues which they nourish. In most instances, the meshes of the capillary network, adjusting themselves to the available free spaces between the elements of the tissues, have a polygonal shape and are of approximately equal size in all planes (Fig. 207).

It is obvious that if the tissue consists of a thin membrane, its capillary network will be arranged in the same plane. If the tissue elements are all elongated and lie parallel to one another, as in nerves, tendons, or muscles, the capillaries between them form a network with elongated meshes, often with right angular meshes in some of the muscles (Fig. 138).

The higher the metabolism of the organ, the denser is its network, and vice versa. In the pulmonary alveoli the meshes are somewhat less than the diameter of the capillary tubes themselves. The capillary networks are dense in various glands, in the mucous membrane of the intestinal tract, etc. In the gray matter of the central nervous system, the vascular network is considerably denser than it is in the white matter which consists only of nerve fibers. In tendons the capil-

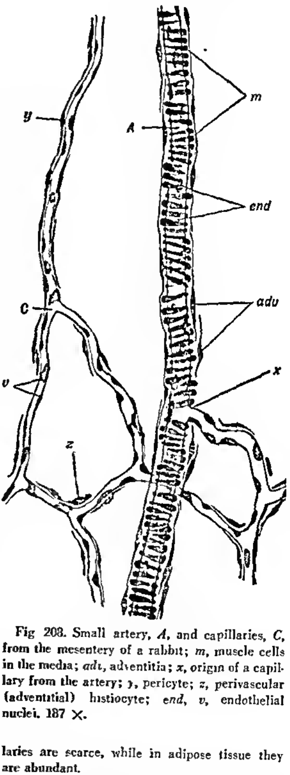

Fig 208. Small artery, *A*, and capillaries, *C*, from the mesentery of a rabbit; *m*, muscle cells in the media; *adv*, adventitia; *x*, origin of a capillary from the artery; *y*, pericyte; *z*, perivascular (adventitial) histiocyte; *end*, *v*, endothelial nuclei. 187 ×.

laries are scarce, while in adipose tissue they are abundant.

The transitions between arteries and capillaries and between capillaries and veins are gradual. This applies to the structure of the wall as well as the caliber of the vessel. However, capillaries are often found which project directly

from small arteries before a complete ramification of the latter has taken place; similarly, accessory capillaries frequently enter directly into a well developed small vein.

In following the ramifications of an artery in the direction of the flow of the blood, only that part of the wall of the vessel which has entirely lost its muscular elements and consists only of endothelium should be called a capillary (Fig. 208, *x*). The first stretches of the capillary network are ordinarily called the *arterial capillaries;* they are usually a little wider than the main mass of the network. Where the veins are formed from the capillary network, the capillary tubes again become gradually wider and at the same time less numerous—these are the so-called *venous capillaries*, which not infrequently have a considerable diameter. The appearance of the first smooth muscle cells or of denser collagenous fibers in their walls indicates the beginning of a small vein. The concept of arterial and venous capillaries is rather indefinite and it is often impossible to distinguish them by the width of their apertures from ordinary capillaries.

In the capillaries of the intestinal villi, part of those in the renal glomeruli, hyaloid membrane of the frog's eye and the majority of developing embryonic capillaries, it has not been possible to demonstrate the outlines of separate endothelial cells. Accordingly, in these places the endothelium is thought to be a continuous, protoplasmic membrane.

Sinusoids. In certain organs there is another type of connection between arteries and veins. These are called "sinusoids" and are structurally quite different from the capillaries. The capillaries have a constant bore and a complete endothelial lining in which the cell boundaries are clearly demonstrable in most cases by treatment with silver nitrate. The sinusoids, on the contrary, have irregular, tortuous walls which vary from 5 to 30 μ or more in diameter in fixed material. Their walls are not formed by a continuous layer of endothelial cells, as in the capillaries, but by irregularly scattered phagocytic and nonphagocytic cells. The ordinary capillary endothelial cells do not store vital dyes or phagocytose bacteria as do the obvious phagocytes of the sinusoids. The outlines of the cell bodies in the sinusoids are not demonstrable in most instances by treatment with silver nitrate. Unlike the capillaries, the sinusoids are not accompanied by a connective tissue layer except for a dense, membranous network of reticular fibrils. The sinusoids probably represent a primitive type of capillary. In the adult mammalian body, sinusoids occur in the blood-forming tissues, the liver and certain endocrine glands.

Some claim that there is no difference between the phagocytes lining the sinuses and the ordinary endothelial cells of other blood vessels and that phagocytosis only occurs in those vessels where the blood flow is supposed to be sluggish—as in the sinuses. However, studies of the living circulation show that the rate of flow varies considerably in all capillaries (and sinuses) and that it often ceases for hours in many vessels which are not sinuses. But phagocytosis and dye storage by the common endothelial cells in these vessels, as in muscle and in the glomeruli, have not been reported. Accordingly, the concept that phagocytosis by cells lining vessels depends on a sluggish circulation should be dropped.

ARTERIES

The blood is carried from the heart to the capillary networks of the tissues and organs of the body by arteries. These are tubes which begin with the aorta and pulmonary artery on the left and right sides of the heart, respectively, and then split into smaller branches. The caliber of the arteries gradually decreases as they recede from the heart, while the sum of the diameters of the lumens of all of the branches of these arteries increases greatly the further they are from the heart. The lining of the walls of all of the arteries consists of the same kind of eodothelium as is found in the capillaries. But external to the endothelium in the arteries, other elements can be distinguished which cause the strength, rigidity, and complexity of the arterial walls; all of these qualities decrease progressively in passing from the larger arteries which originate in the heart to the capillaries.

Besides the endothelium the arteries are composed of: (1) fibroblasts and collagenous fibers, (2) bands and networks of elastic fibers, and (3) smooth muscle cells. The arterial walls are abundantly provided with nerves, and in the large arteries small blood and lymphatic vessels are present.

The wall of the largest arteries (as the aorta) differs from the wall of arteries of medium caliber (as the radial artery) by its absolute thickness and by its structure. In the aorta the middle layer of the vessel is distinctly yellow, due to the predominance of elastic elements; in the second instance it is red gray because of smooth muscles. The arteries of *elastic type* are of large caliber and include the aorta, innominate, subclavian, the beginning of the common carotid, and the pulmonary arteries; these are also called the *conducting arteries.* The arteries of *muscular type* (the *distributing arteries*) include the majority of the arteries; they continue from the above-mentioned large vessels close to the heart and extend to the unnamed arteries which are difficult to distinguish with the naked eye. The smallest arteries, 0.3 mm. in diameter or smaller, are usually grouped in a separate class and are called *arterioles.* In the transition between the arterioles and the capillaries some authors distinguish *precapillary arterioles*—an ill-defined concept. The peculiarities in the structure of the different types of arteries are reflected in their physiologic significance.

In the walls of every artery, three layers can be distinguished: (1) The inner coat, *tunica intima* or *interna,* whose elements are oriented mainly longitudinally. (2) The intermediate coat, *tunica media;* most of its elements are directed circularly. This is the thickest layer of the wall and its character determines the type of artery. (3) The external coat, *tunica adventitia* or *externa;* most of its elements run parallel to the long axis of the vessel. The elements of this tunic gradually merge with those of the surrounding loose connective tissue which accompanies every blood vessel. The boundary between the tunica intima and tunica media is formed by the *internal elastic membrane* which is particularly noticeable in arteries of medium caliber. Between the tunica media and the

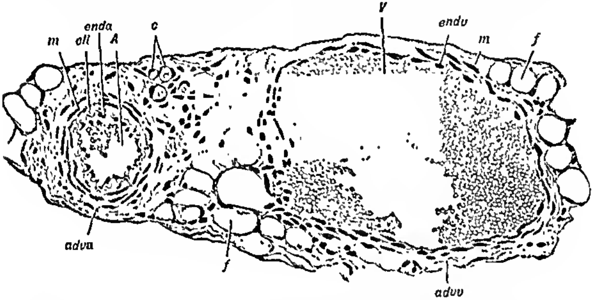

Fig. 209. Cross section through a small artery (*A*) and its accompanying vein (*V*) from the submucosa of a human intestine: *adva* and *advv,* Adventitia of the artery and vein; *c,* cross sections of capillaries; *enda* and *endv,* endothelium of the artery and vein; *eli,* elastica interna; *m,* muscle cells of the media; *f,* fat cells in the loose connective tissue. 187 ×. (A.A.M.)

tunica adventitia, an external elastic membrane can be distinguished in most cases. agonal contraction of the muscle fibers throws it into longitudinal folds.

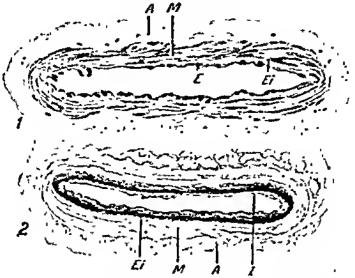

Fig. 210. Cross section of the central artery of the human retina: *1*, Stained with hemalum-eosin; *2*, stained with orcein; *A*, adventitia; *E*, endothelium; *Ei*, internal elastic membrane; *I*, intima; *M*, muscular layer. 160 ×. After Schaffer.

Small Arteries—Arterioles. The *tunica intima* consists only of endothelium and the internal elastic membrane (Fig. 209). In cross section the elastica interna usually appears as a thin, bright line just beneath the endothelial nuclei. It is markedly scalloped in sections, because the

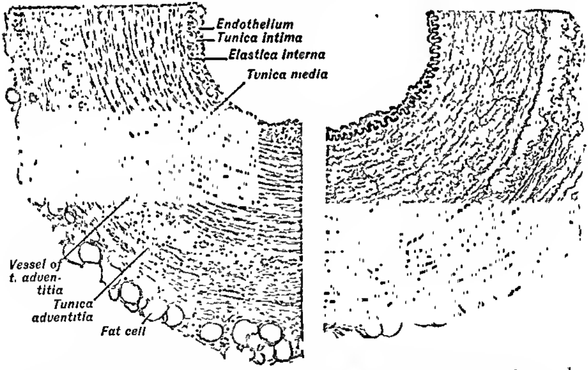

Fig. 211. Two cross sections of the same volar digital artery of a man; left, stained with hematoxylin and eosin; right, stained with orcein to show elastic tissue. 80 ×. Slightly modified after Schaffer.

The *tunica media* of small arteries consists of smooth muscle cells, 15 to 20 μ in length. They are always oriented transversely to the length of the vessel, and are bent with the curvature of the arterial wall. The number of layers of muscle cells depends on the caliber of the artery.

The *tunica adventitia* approximately equals the tunica media in thickness; it is a layer of loose connective tissue with longitudinally oriented, collagenous and elastic fibers, and a few fibroblasts. It merges with the surrounding connective tissue. The small arteries lack a definite external elastic membrane.

When these small arteries merge into capillaries, the endothelium remains uninterrupted and unchanged while the internal elastic membrane becomes progressively thinner and disappears when the vessel has a diameter of 62 μ. The spindle-shaped muscle cells disappear when the caliber of the blood vessel decreases to that of the capillary. The tunica adventitia loses its elastic fibers which are replaced by networks of reticular fibers and the perivascular cells of the capillaries.

Arteries of Medium Caliber or of Muscular Type. This group comprises most of the arteries. The *tunica intima* is lined by the endothelium which is continuous with that of the arteries of small caliber. Beneath the endothelium in the smaller arteries of this group is the internal elastic membrane. In larger vessels, collagenous and elastic fibers and a few fibroblasts lie beneath the endothelium.

The *internal elastic membrane* is well developed. In cross section it appears homogeneous and bright; due to agonal contraction of the tunica media it is typically scalloped (Fig. 211). In large blood vessels it is a thick, "fenestrated" elastic membrane provided with a number of irregular, rounded or oval openings (Fig. 214). In many arteries the elastica interna is split into two or more layers (Fig. 212, *I*).

The *tunica media* of arteries of muscular type consists almost exclusively of smooth muscle cells arranged in concentric layers. Thin reticular fiber membranes can be demonstrated to form sheaths for the individual muscle cells. Thin elastic fiber networks with wide meshes course circularly in the tunica media and continue into the external and internal elastic membranes (Fig. 212, *G*).

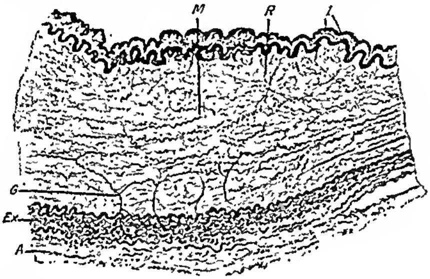

Fig. 212. Portion of a cross section of a mesenteric artery of a man. *A*, Adventitia; *Ex*, external elastic layer; *G*, branching elastic fibers; *I*, elastica of the intima, which is here split into two layers; *M*, tunica media; *R*, radial elastic fibers. Orcein stain. 110 ×. After Schaffer.

The *tunica adventitia* of arteries of the muscular type is sometimes thicker than the tunica media. It consists of loose connective tissue whose collagenous and elastic fibers pass predominantly in the longitudinal or tangential directions. The elastic layer immediately adjacent to the smooth muscles stands out as a well defined, perforated membrane, the external

elastic membrane. The tunica adventitia passes over into the surrounding connective tissue without sharp boundaries. Owing to this loose consistency of its external

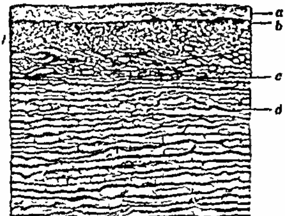

Fig. 213. Longitudinal section through the posterior wall of the human descending aorta. The elastic tissue is black—the other elements are not shown clearly. *I*, Intima; *M*, media; *A*, adventitia; *a*, subendothelial layer; from *b* to *c*, the longitudinally striated layer, which becomes a fenestrated membrane at *c*; *d*, fenestrated elastic membrane; *e*, the last membrane, elastica externa, on the boundary between adventitia and media; *vv*, vasa vasorum. Elastic fiber stain. 85 ×. Redrawn after Kölliker-v. Ebner.

layers, the tunica adventitia permits the arteries to move within certain limits and allows the constant changes in the size of the lumen; it also limits the amount of shortening of the arteries which takes place after they are cut.

Arteries of Large Caliber or of Elastic Type. The resistant, elastic wall of these blood vessels (as the aorta) is much thinner in comparison with the size of their lumen than that of the vessels of the preceding group.

Tunica Intima. The tunica intima in an adult man is rather thick (127 μ) (Fig. 213, *I*). The endothelium differs from the endothelium in smaller arteries by the fact that the cells are not elongated but have an oval or polygonal form. The layer directly beneath the endothelium is normally thin. It consists of a few thin interlacing fibers and fibroblasts. A few wandering cells may be present normally. The next layer consists of many branching elastic fibers which fuse in places into a more or less well pronounced, striated membrane. Between these fibers are a few collagenous fibers, fibroblasts, and small bundles of smooth muscle cells. Externally this layer of elastic fibers passes into a fenestrated, elastic membrane, which by its location corresponds with the internal elastic membrane. But it does not differ from the many similar membranes which follow toward the exterior and form the tunica media of the aorta. Thus, the tunica intima in the largest blood vessels is but poorly delimited from the tunica media.

Tunica Media. The tunica media consists *mainly* of elastic tissue (Fig. 213, *M*). In the human aorta it appears in the form of 50 to 65 concentric "fenestrated" elastic membranes, 2.5 μ thick, between which the interspaces measure 6 to 18 μ. The neighboring membranes are frequently connected with one another by elastic fibers or bands.

In the spaces between two adjacent elastic membranes are thin layers of connective tissue with very thin collagenous and elastic fibers, fibroblasts, and smooth muscle cells (Fig. 215). The latter, particularly in the inner layers of the tunica

media of the aorta, are flattened, branched elements with very irregular outlines and serrated edges; they have characteristic, rodlike nuclei. Most of them are arranged circularly. These smooth muscle cells are closely surrounded by collagenous fibers which bind them to the elastic membranes. Between these various structures is an appreciable amount of basophil amorphous ground substance which stains like mucus with certain dyes. The basophilia of this ground substance is believed by some to be due to the presence of chondroitin sulphuric acid.

Tunica Adventitia. The tunica adventitia in arteries of large caliber is relatively thin. It cannot be sharply distinguished from the surrounding connective tissue. The most external of the fenestrated membranes of the tunica media serves as an external elastic membrane, from which numerous elastic fibers project. There is a gradual transition from the tunica adventitia into the surrounding loose connective tissue with its fat cells.

The Connection Between Arteries of Different Types. As one type of artery goes over into another without marked boundaries, it is sometimes very difficult to classify an artery as of a given type. Some arteries of rather small caliber (popliteal, tibial) have walls which suggest large arteries, while some large arteries (external iliac) have walls like those of medium-sized arteries. The change of an artery of elastic type into an artery of muscular type usually takes place gradually, so that the intermediate regions are often designated *arteries of mixed type*. Such are the external carotid, axillary, and common iliac arteries. In their middle tunics are islands of smooth muscle fibers which interrupt the elastic membranes in many places.

Where arteries of mixed or elastic type pass suddenly into arteries of the muscular type, short transition regions occur; these are called *arteries of hybrid type*, and are found in the visceral arteries which arise from the abdominal aorta (Fig. 212). In them, for a varying distance, the tunica media may consist of two different layers—the internal is muscular and the external is composed of typical elastic membranes.

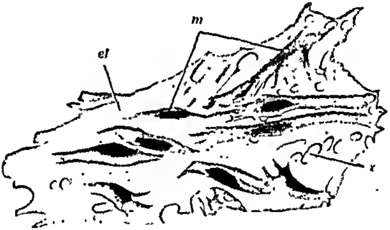

Fig. 214. Portion of a fenestrated elastic membrane from the aorta of a calf: *el*, Elastic substance; *x*, openings; *m*, smooth muscle cells adherent to the membrane. 250 ×. Redrawn after Prenant.

Special Types of Arteries. In the tunica media of the arteries of the lower limbs, the muscular tissue is more highly developed than in the arteries of the upper limbs.

The *arteries of the skull*, which are protected from external pressure or tension, have a very thin wall and a well developed elastica interna. In the tunica media the elastic fibers are almost entirely absent.

The *umbilical artery* has a quite atypical, special structure. Its intima consists only of endothelium and lacks an internal elastic layer. The tunica media contains a small number of elastic

fibers and two thick, muscular layers which are sharply separated from each other. The inner layer is composed of longitudinally directed fibers; in many places these form longitudinal protrusions into both the lumen and the outer circular muscular layer. The extra-abdominal portion of the umbilical artery is provided with numerous oval swellings; in these regions the wall becomes thin and consists almost exclusively of circularly arranged muscles. The various organs show differences in their arteries. These are described in the following chapters.

Physiologic Significance of the Structure of Arteries. As the movement of the blood in the arteries is caused by the contractions of the heart, it is rhythmically interrupted. If the walls of the arteries were inflexible, the flow of blood in their terminal branches would also be irregular. But the walls of the largest vessels near the heart are composed of an elastic, easily expanding tissue, and only a part of the force of contraction (systole) immediately advances the blood. The remainder of the force of the contraction expands the large elastic arteries and is accumulated as potential energy in the increased elastic tension of the arterial wall. With the closure of the aortic and pulmonary valves this tension becomes transformed into kinetic energy which moves the blood forward while the ventricles are at rest (diastole). At the beginning of the arterial system the flow of blood is irregular; it becomes more and more continuous in the direction of the terminal ramifications.

The arteries of elastic type can be regarded as regulating the general blood circulation, while the muscular arteries, by contracting or relaxing, decrease or increase the supply of arterial blood in any region of the body. These contractions and dilatations of the muscular arteries are regulated by the *vasoconstrictor* and *vasodilator nerves* of the autonomic nervous system which terminate in the smooth muscles.

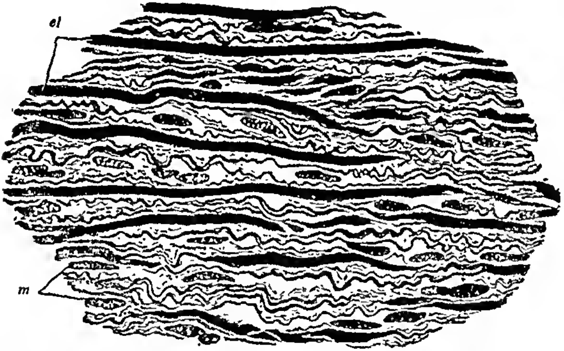

Fig. 215. Cross section of a part of the media of the aorta of a five-year-old boy: *el*, Cross sections of the fenestrated elastic membranes, between which are fine collagenous fibers; *m*, smooth muscle nuclei. Orcein and hematoxylin. 500 ×. (A.A.M.)

The muscular tissue of the arterial walls is normally somewhat *contracted;* this is the basis of the tone of the vessels. The degree of tone fluctuates continuously.

Changes in the Arteries with Age. The arterial blood vessels reach their mature form only

in adult life. During the fourth month of embryonic life in man, the arteries first acquire their three main layers, tunica intima, tunica media, and tunica adventitia. From this time the wall of the vessels changes gradually, so that the intima of the aorta, for example, becomes complete only at about thirty years of age. The arterial system, with the heart, is always active mechanically and seems to wear out more than any other system of organs. Accordingly, the final differentiation of the structure of the wall frequently cannot be sharply separated from the regressive changes which develop gradually with age and lead to *arteriosclerosis*. Indeed, some authors view this process as a physiologic, others as a pathologic, progression. In general, arteriosclerosis is a pathologic process when its intensity in a given vessel is beyond the norm for this vessel at a particular age The arteries of elastic type, particularly the aorta, show much greater changes with age than do the arteries of muscular type. The small arteries hardly participate in this process under physiologic conditions.

In the aorta of a four months' human embryo, the intima consists only of the endothelium and of one rather thick, elastic membrane—the elastica interna. The media consists of several layers of circular smooth muscles, between which are flat networks of elastic fibers. The adventitia is thicker than the media and consists of embryonic connective tissue. See also Jackson (1935).

At the end of embryonic life the internal elastic membrane becomes thicker while the flat networks of elastic fibers in the media turn into thick elastic membranes The muscular elements have increased slightly in number, but are still inconspicuous. The adventitia by this time has become smaller.

After birth the number and thickness of the elastic membranes in the media of the aorta gradually increase. They are much like the elastica interna now. Between the endothelium and the elastica interna in the intima, an elastic muscular layer appears. It arises in part by a splitting of the elastica interna and in part by the new formation of collagenous and elastic fibers, and gradually increases in thickness. At about the age of twenty-five, these layers are completely differentiated

The medium-sized muscular arteries, as the brachial, even in the middle of embryonic life, have an intima composed of an endothelium and an elastica interna, a media of circular smooth muscles, and an adventitia The last has a well pronounced elastica externa which is surrounded by a connective tissue layer rich in elastic fibers Toward the end of the embryonic period the greatly thickened media consists only of circular muscles bounded by the external and internal elastic membranes. After birth, in the arteries of muscular type, in addition to the thickening of the wall as a whole, a connective tissue layer gradually develops between the endothelium and the elastica interna.

The wearing out of a large vessel, as the aorta, is shown mainly in an irregular thickening of the tunica intima. Later on, fat infiltrates the interstitial substance and the degenerative processes begin. In the tunica media, the elastin of the fenestrated membrane may transform into the nonelastic *elacin*. In the medium-sized arteries of muscular type, the main change is a calcification within the tunica media, although the intima frequently thickens through a splitting of the elastica interna and the new formation of collagenous and elastic fibers.

VEINS

The blood is carried from the capillary networks toward the heart by the veins. In progressing toward the heart, the caliber of the veins gradually increases while the wall becomes thicker. The veins usually accompany their corresponding arteries. As the caliber of an artery is always less than that of the corresponding vein or veins, the venous system has a much greater capacity than the arterial (Fig. 209). The wall of the veins is always thinner, softer and less elastic than that of the arteries. Hence in sections the veins, if empty, are collapsed and their lumen is irregular and slitlike.

One can frequently distinguish three types of veins: those of small, medium-sized and large calibers. This subdivision is often unsatisfactory for the caliber and the structure of the wall cannot always be correlated. Individual veins show much greater variations than do the arteries, and the same vein may show great differences in different parts.

Most authors distinguish three layers in the walls of the veins; tunica intima, tunica media and tunica adventitia. But their boundaries are frequently indistinct and in certain veins these coats, particu-

larly the tunica media, cannot be distinguished. The muscular and elastic tissue is much more poorly developed in the veins than in the arteries, while the connective tissue is much more prominent in the veins.

Veins of Small Caliber. When several capillaries unite they first form a tube about 20 μ in diameter. This consists of a layer of endothelium surrounded by a very thin layer of longitudinally directed collagenous fibers and fibroblasts (Fig. 216). When the caliber has increased to about 45 μ, partially differentiated smooth muscle cells appear between the endothelium and the connective tissue. These cells are at first located at some distance from one another; they later become arranged closer and closer together. In veins with a diameter of 200 μ these elements form a continuous layer and have a typical, long, spindle shape. In still larger veins thin networks of elastic fibers appear. In them the tunica intima consists only of endothelium while one or several layers of smooth muscle cells form the media. The tunica adventitia consists of scattered fibroblasts and thin elastic and collagenous fibers; most of them run longitudinally and some enter the spaces between the muscle cells of the tunica media.

Veins of Medium Caliber. The veins of medium caliber (2 to 9 mm.) include the cutaneous and deeper veins of the extremities up to the brachial and the popliteal, and the veins of the viscera and head with the exception of the main trunks. In the *tunica intima* of these veins the endothelial cells are irregular polygons. Sometimes the tunica intima also contains an inconspicuous connective tissue layer with a few cells and very thin elastic fibers. Externally it is sometimes bounded by a network of elastic fibers. As it is frequently feebly developed, some authors consider the inner and middle coats as forming one layer.

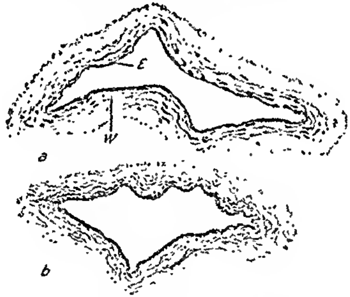

Fig. 216. Cross section through the central vein of the human retina. *a*, Stained with hemalum-eosin; *b*, with acid orcein. *E*, Endothelium; *W*, connective tissue wall whose fine elastic fibers are visible in *b*. 160 ×. After Schaffer.

The *tunica media* is much thinner than in the arteries, and consists mainly of circular smooth muscle fibers separated by many longitudinal collagenous fibers and a few fibroblasts (Fig. 217).

The *tunica adventitia* is usually much thicker than the media and consists of loose connective tissue with thick, longitudinal, collagenous bundles, and elastic networks. It often contains in the layers

adjacent to the media a number of longitudinal smooth muscle bundles.

The *tunica media* in general is poorly developed and is sometimes absent. Its structure is the same as in the veins of medium caliber. The *tunica adventitia* composes the greater part of the venous wall and is usually several times as thick as the tunica media (Fig. 218). It con-

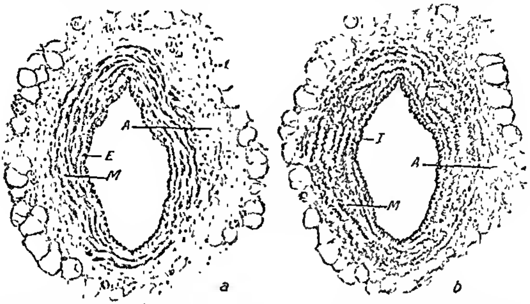

Fig. 217. Cross section through the common digital vein of a man *a*, Stained with hemalum-eosin; *b*, with acid orcein; *A*, adventitia; *E*, endothelium; *I*, internal elastic membrane; *M*, muscular coat. 80 ×. After Schaffer.

Veins of Large Caliber. The *tunica intima* has the same structure as in the medium-sized veins. In some of the larger trunks its connective tissue layer is of considerable thickness (45 to 68 μ).

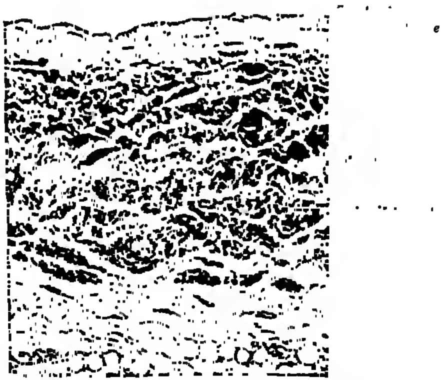

Fig. 218 Low power view of human vena cava. Note the muscular adventitia.

sists of loose connective tissue containing thick elastic fibers and mainly longitudinal collagenous fibers. In the layer adjacent to the tunica media or, if the latter is absent, to the tunica intima, the tunica adventitia contains prominent longitudinal layers of smooth muscles and elastic networks. This is the structure of the inferior vena cava and the portal, splenic, superior mesenteric, external iliac, renal and azygos veins.

like an artery in this respect. Smooth muscles are particularly prominent in all the layers of the walls of the veins in a pregnant uterus.

Certain veins are entirely devoid of smooth muscle tissue and consequently of a tunica media. In this group belong the veins of the maternal part of the placenta, of the spinal pia mater, of the retina, of bones, the sinuses of the dura mater, the majority of the cerebral veins, the veins of the nail bed and of the trabeculae of the spleen. The last two are simply channels lined by endothelium with a fibrous connective tissue covering.

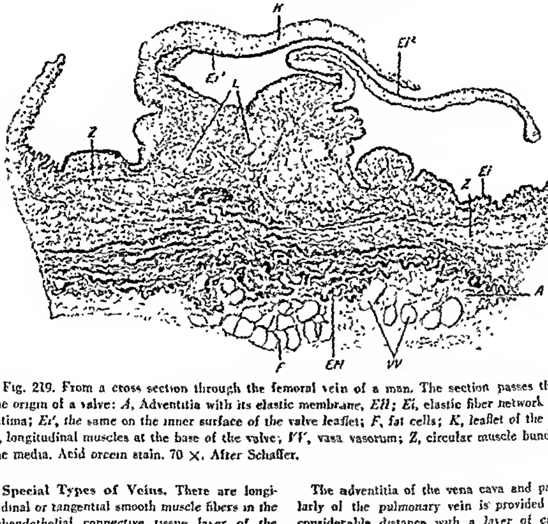

Fig. 219. From a cross section through the femoral vein of a man. The section passes through the origin of a valve: *A*, Adventitia with its elastic membrane, *EH*; *Ei*, elastic fiber network in the intima; *Ei'*, the same on the inner surface of the valve leaflet; *F*, fat cells; *K*, leaflet of the valve; *L*, longitudinal muscles at the base of the valve; *VV*, vasa vasorum; *Z*, circular muscle bundles in the media. Acid orcein stain. 70 ×. After Schaffer.

Special Types of Veins. There are longitudinal or tangential smooth muscle fibers in the subendothelial connective tissue layer of the tunica intima of the iliac, femoral, popliteal, saphenous, cephalic, basilar, median, umbilical, and other veins. In certain veins, the longitudinal orientation is also noticed in the innermost muscular layers of the tunica media.

In a considerable portion of the inferior vena cava, the tunica media is absent and the well developed longitudinal muscle bundles of the tunica adventitia are directly adjacent to the intima. In the pulmonary veins the media is very well developed with circular muscles and is

The adventitia of the vena cava and particularly of the pulmonary vein is provided for a considerable distance with a layer of cardiac muscle fibers arranged in a ring with a few longitudinal fibers where these vessels enter the heart. In the rat, the pulmonary veins up to their radicles contain much cardiac muscle in the tunica media.

The Valves of the Veins. Many veins of medium caliber, particularly those of the extremities, are provided with valves which prevent the blood from flowing away from the heart. These are semi-

lunar pockets on the internal surface of the wall and are directed with their free edges in the direction of the blood flow. In man they are usually arranged in pairs, one opposite the other, distal to the branches entering the veins. Between the valves and the wall of the veins there is the so-called *sinus of the valve*; in this place the wall of the blood vessel is usually distended and quite thin.

The valve is a thin, connective tissue membrane; on the side toward the lumen of the vessel, it contains a thick network of elastic fibers which are continuous with those of the tunica intima of the vein (Fig. 219, *Ei*). The wall of the vein is thinner in the region of the sinus; here its intimal and medial tunics contain only longitudinal smooth muscles; these do not enter into the substance of the valve in man (Fig. 219, *L*).

Both surfaces of the valve are covered by endothelium which is reflected from the internal surface of the intima. The endothelial cells lining the surface toward the lumen of the vessel are elongated in the axis of the vessel; those which line the valves facing the sinus, on the other hand, are elongated transversely.

The Blood Vessels of Blood Vessels (Vasa Vasorum). The walls of all arteries and veins with a caliber greater than 1 mm. are provided with their own blood vessels, the vasa vasorum. These originate from the adjacent small arteries and form a dense capillary network in the adventitia. In even the larger arteries they do not penetrate further than the external layers of the media (Fig. 213, *vv*). In the veins, however, they are in general more abundant and may even penetrate up to the intima; the veins of these blood vessels often open into the lumen of the vessels which they drain. Networks of thin-walled, frequently very wide lymphatics have been proved to be present in all of the larger arteries and veins. They connect with the *perivascular lymphatics* and according to some authors may even be traced into the media. The blood vessels in the central nervous system are surrounded by perivascular lymphatic spaces which are bounded externally by a limiting membrane of neuroglia

Nerves of Blood Vessels. All the blood vessels, particularly the arteries, are well supplied with nerves. These are of two types, vasomotor and receptor or sensory nerves. (See p. 198.)

Other Connections Between Arteries and Veins. As a general rule, a capillary network connects the terminal ramifications between the arteries and veins and the transition occurs gradually. In many organs and tissues, however, there are modifications of this vascular plan which are adapted to the peculiar functions of the particular tissues.

In certain cases an artery or vein may ramify into a number of capillaries which are then collected into larger vessels of the original type, *i. e.*, an artery or vein. An example of this is found in the arteries which form the glomeruli of the kidney; the afferent artery suddenly breaks up into a mass of twisting capillaries which coalesce to form the efferent artery. The portal vein of the mammalian liver arises from the capillary networks of the abdominal viscera, enters the liver and separates into a network of sinusoids. They penetrate the organ and are then gathered into the hepatic vein. This is a "portal" system.

Arterio-venous Anastomoses. The terminal ramifications of arteries are connected with veins not only by capillaries but also by direct arterio-venous anastomoses in many parts of the body. As the lumen of these anastomoses changes within wide limits and is often closed, the anastomoses are probably a mechanism for the local regulation of blood circulation and pressure. In addition to these simple direct communications, Masson has described highly organized connections between arteries and veins which occur as part of a specific organ, the *glomus*, found in the nail bed, the pads of the fingers and toes, ears, hands and feet. The afferent arteriole enters the connective tissue capsule of the glomus, loses its internal elastic membrane and develops a heavy epithelioid muscle coat and narrow lumen. This arterio-venous anastomosis of the glomus may be branched and convoluted, and is richly innervated by sympathetic

and myelinated nerves. The anastomosis empties into a short, thin-walled vein with a wide lumen which drains into a periglomic vein and then into the ordinary veins of the skin.

In addition to helping regulate the flow of blood in the extremities, it is claimed that the glomus is concerned with temperature regulation and conservation of heat.

A special place in the blood vascular system is occupied by the cavernous tissue of erectile organs (see Chapter XXIV).

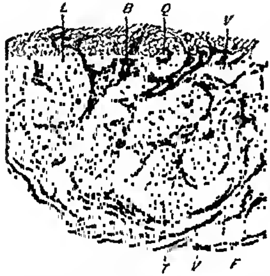

Fig. 220. Coccygeal body of man. Portion of a cross section: B, Connective tissue; F, fat cells; L, vessel in longitudinal, Q, in cross, and, T, in tangential sections; V, veins. Mallory's aniline blue stain. 110 ×. After Schaffer.

The Coccygeal Body. This organ, erroneously included in the paraganglia, does not contain chromaffin cells. It is situated in front of the apex of the coccyx and measures 2.5 mm. in diameter. It consists of numerous arterio-venous anastomoses embedded in a dense fibrous matrix. The smooth muscle cells have undergone extensive "epithelioid" change. An internal secretion has not been demonstrated in this organ.

THE HEART

The heart, a thick, muscular, rhythmically contracting portion of the vascular system, is a roughly conical organ. It lies in the pericardial cavity within the mediastinum. It is about 12 cm. long, 9 cm. wide, and 6 cm. in its anteroposterior diameter. It consists of four main chambers: a right and left *atrium* and a right and left *ventricle*. The superior and inferior venae cavae bring the venous blood from the body to the right atrium whence it passes to the right ventricle. From here the blood is forced through the lungs where it is aerated and brought to the left atrium. It then passes to the left ventricle and is distributed throughout the body by the aorta and its branches. The atria are separated from the ventricles by the tricuspid and mitral valves on the right and left sides. The pulmonary artery and the aorta are separated from the right and left ventricles respectively by the semilunar valves.

The wall of the heart, in both the atria and the ventricles, consists of three main layers: (1) the internal or *endocardium*, (2) the intermediate or *myocardium*, (3) the external or *epicardium*. The internal layer is in immediate contact with the blood; the myocardium is the contractile layer; and the epicardium is the visceral layer of the *pericardium*. This is a serous membrane which forms the pericardial sac in which the heart lies.

Most authors believe that the endocardium is homologous with the tunica intima of the blood vessels, the myocardium with the tunica media, and the epicardium with the tunica adventitia.

Endocardium. The endocardium is lined with ordinary endothelium which is continuous with that of the blood vessels entering and leaving the heart. This endothelium consists of rounded or polygonal cells. In most places, directly under the endothelium, there is a thin *subendothelial layer;* it contains collagenous and a few elastic fibers and fibroblasts. External to this layer is a thick layer of connective tissue which composes the main mass of the endocardium and contains great num-

bers of elastic elements (Fig. 221, *G*). In the left atrium these elastic fibers pass into a typical, fenestrated, elastic membrane. Bundles of smooth muscle fibers are found in varying numbers in this layer, particularly on the interventricular septum (Fig. 221, *L*).

A *subendocardial layer* (Fig. 221, *I*), absent from the papillary muscles and the chordae tendineae, consists of loose connective tissue which binds the endocardium and the myocardium together and is directly continuous with the interstitial tissue of the latter. It contains blood vessels, nerves, and branches of the conduction system of the heart. In the spaces between the muscular bundles of the atria, the connective tissue of the endocardium continues into that of the epicardium and the elastic networks of both layers intermingle.

Myocardium. The minute structure of the cardiac muscle has been described on page 171. In the embryos of the higher vertebrates the myocardial fibers form a spongy network. In the adult stage, however, they are bound by connective tissue into a compact mass. This condensation of the myocardium progresses from the epicardium toward the endocardium. Many embryonic muscular bars remain in a more or less isolated condition on the internal surface of the wall of the ventricular cavities. They are covered with endocardium and are called "trabeculae carneae."

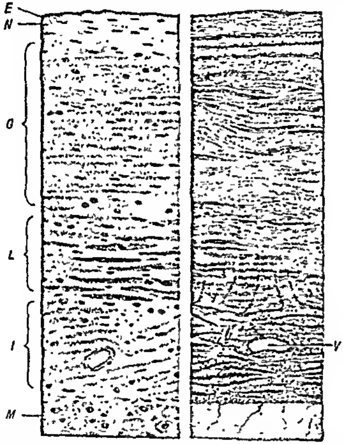

Fig. 221. Cross section through the left atrium of a man: to the left after staining with hematoxylin and eosin, to the right after orcein. *E*, Endothelium; *N*, subendothelial layer; *G*, inner layer, and, *L*, outer layer with smooth muscle fibers of the endocardium; *I*, subendocardial layer; *M*, myocardium; *V*, blood vessel. 200 × Redrawn after Favaro.

Elastic elements are very scarce in the myocardium of the ventricles of adult mammals except in the tunica adventitia of the larger blood vessels of these chambers In the myocardium of the atria, however, there are networks of elastic fibers which run everywhere between the muscle fibers and are directly connected with similar net-

works in the endocardium and epicardium. They are also continuous with the elastic networks in the walls of the large veins. A large part of the interstitial connective tissue of the cardiac muscle consists of extensive networks of reticular fibrils.

Epicardium. The epicardium is covered on its free surface by a single layer of mesothelial cells. Beneath the mesothelium is a thin layer of connective tissue with flat networks of elastic fibers, blood vessels and many nervous elements. About the adventitia of the coronary vessels there is a loose layer of considerable thickness which contains much adipose tissue.

The parietal layer of the pericardium is a serous membrane of the usual type—a flat layer of connective tissue which contains elastic networks, collagenous fibers, fibroblasts, fixed macrophages, and a covering layer of mesothelial cells. Removal of the parietal pericardium in cats results in a great thickening of the epicardium and an enlargement of the heart.

The Cardiac Skeleton. The central supporting structure of the heart, to which most of the muscle fibers are attached and with which the valves are connected, is the cardiac skeleton (*Poirier, Tandler*). It has a complicated form and consists mainly of dense connective tissue; its main parts are the *septum membranaceum*, the *trigona fibrosa*, and the *annuli fibrosi* of the atrioventricular and the arterial foramina.

In man the fibrous rings consist mainly of dense connective tissue which contains some fat and thin elastic fibers. The structure of the septum membranaceum suggests that of an aponeurosis, with its more regular distribution of collagenous bundles in layers. The connective tissue of the trigona fibrosa contains islands of chondroid tissue. The cells of the latter are globular as in cartilage, although they lack true capsules. The interstitial substance stains deeply with basic aniline dyes and hematoxylin, and is penetrated by collagenous fibers and practically no elastic fibers. In aged individuals the tissue of the cardiac skeleton may in places become calcified and sometimes even ossified.

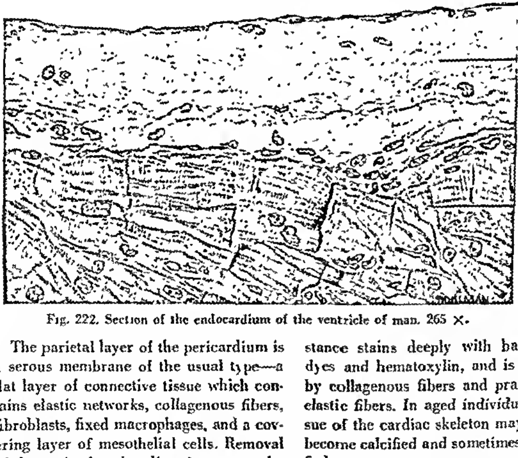

Fig. 222. Section of the endocardium of the ventricle of man. 265 ×.

There are important differences in the histologic structure of the cardiac skeleton among different animals, and even in individuals of different ages. In some cases it is a simple, dense connective tissue with a few elastic fibers and is directly continuous with the interstitial tissue of the myocardium; in some cases it approaches cartilage in its structure (horse and pig); in the dog it forms true hyaline cartilage, and contains

bone in the ox. These different types of tissue may be located in islands side by side, and one type may merge into another.

The Cardiac Valves. *Atrioventricular Valves.* These consist of a plate of connective tissue which begins at the annulus fibrosus and is reinforced by ligamentous threads. It is covered on the atrial and ventricular sides by a layer of endocardium. At the free edge of the valve these three layers blend.

The ground plate consists mainly of dense chondroid tissue with small, spindle-shaped or rounded cells and a basophil, fibrillated, interstitial substance. The endocardial layer is thicker on the atrial side. Here the subendothelial layer has a small amount of chondroid tissue and rests upon a connective tissue layer which contains many elastic fiber networks and some smooth muscles. In the vicinity of the annulus fibrosus the subendocardial layer is quite loose and the musculature of the atrium penetrates far into it. On the ventricular side the endocardial layer has a similar structure but is much thinner. In many places the chordae tendineae enter it, and at the base of the valves are some muscle fibers from the ventricle.

Aortic and Pulmonic Valves. The aortic and pulmonic valves have the same general structure as the atrioventricular valves. In the middle of the valve are plates of chondroid tissue with collagenous and thin elastic fibers. At the root of the valve all of these continue into the annulus fibrosus of the arterial foramen and at the middle of the free edge they form the noduli Arantii.

On the arterial side this plate is covered with a thick, uneven endocardium consisting of: (1) connective tissue with very coarse, collagenous bundles (Fig. 224, *IIIa*) and a few elastic fibers; (2) a thin subendothelial layer with an elastic network and a peripheral endothelium (Fig. 224, *IIIb*). On the ventricular side the central plate (Fig. 224, *II*) is covered with a thick endocardium composed of: (1) a connective tissue layer with longitudinal collagenous and elastic fibers (Fig. 224, *Ic*) and (2) two connective tissue layers which are not sharply outlined from each other; one of these contains longitudinal (Fig 224, *Ia*) and the other transverse (Fig. 224,

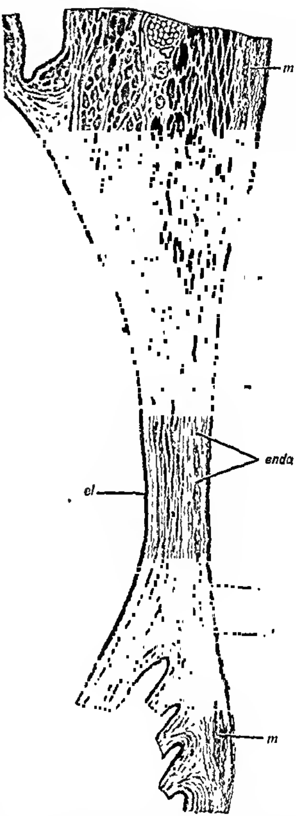

Fig. 223 Cross section through the mitral valve of man. Atrial surface on the right, ventricular on the left. In the upper left-hand corner is the attachment of the aortic valve; on the left, below, is the passage of chordae tendineae into the valve: *A*, Dense tissue plate; *enda*, endocardium from the atrial, and, *endv*, from the ventricular side; *el*, elastic fibers; *m*, myocardium. Low magnification Redrawn after Sato.

(b) elastic fibers; the covering is endothelium. There is here also a dense network of particularly thick, elastic fibers which suggest the elastica interna of arteries.

The Impulse Conducting System. In the vertebrate embryo and in the adult lower vertebrates, the heart is a bent tube whose contractile walls are dilated and constricted in several places. The tube consists of four portions which lie behind one another in the caudocranial direction: (1) Sinus venosus, (2) atrium, (3) ventricle, (4) conus arteriosus. Patten and Kramer studied living chick embryos and noted that the heart develops by "progressive fusion of paired primordia, ventricular end first, then atrium, and last of all sinus." As each part forms it begins to beat and controls the rate of contraction of the parts previously laid down. Thus, the regular activity of a primitive vertebrate heart is based on: (1) the origin of the stimulus in a definite area of the organ, and (2) the transmission of this stimulus to the following portions

In the adult mammalian heart, too, the motor impulse arises in that part of the heart which develops from the embryonic sinus venosus, that is, where the superior vena cava enters the right atrium, and there is a specialized mechanism by which the contraction spreads to the atria and then to the ventricles.

Beginning with the sinus node and extending up to the papillary muscles and the other portions of the myocardium of the ventricles, there is a continuous tract of atypical muscles (the Purkinje fibers, p. 174), the *sinoventricular system.* This system serves for the origin and transmission of the contractile impulse. This conduction system is accompanied by many nerves which also play a part in carrying the contractile impulse. The usual descriptions picture the Purkinje fibers as arising at the sinus node, spreading over the atria, concentrating at the atrioventicular node, and passing by one main bundle (the atrioventricular bundle) to the ventricles, where they again branch out over the whole inner surface of these cavities. This description is but partially correct. The atypical fibers of the conduction system do appear in these positions; in addition, however, they pass from the atria to the ventricles by several other routes which have not been studied as thoroughly as the atrioventricular bundle.

This system of conduction fibers, even up to the terminal ramifications in the ventricles, is covered with a connective tissue membrane which separates it from the remaining muscular mass of the heart.

At the boundary between the right atrium and the superior vena cava, in the region of the sulcus terminalis, is the sino-atrial node, 1 cm. in length

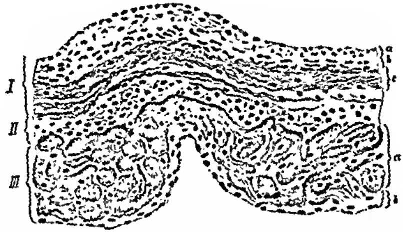

Fig. 221 Section through a human aortic valve. Above is the ventricular surface; below is the lumen of the aorta. Elastic fibers unstained. See text, p. 249, for detailed explanation. Redrawn from Mönckeberg.

and 3 to 5 mm. in width; although not sharply outlined, it can be seen with the naked eye. It consists of a dense network of twisted Purkinje fibers.

The atrioventricular node is a flat, white structure about 6 mm. long and 2 to 3 mm. wide; it is located in the posterior lower part of the interatrial septum under the posterior aortic valve. The node consists of Purkinje fibers which form a tangled dense network whose meshes are filled with connective tissue. These fibers pass into (or between) the usual myocardial fibers, so that the boundary of the node is very indistinct over much of its periphery. Toward the ventricles the substance of the node contracts abruptly into a shaft about 1 cm. long, the atrioventricular bundle; it is located in the dense connective tissue of the trigonum fibrosum dextrum and continues into the septum membranaceum where it divides into two branches.

The first branch, a cylindrical bundle 1 to 2 mm. thick, runs downward along the posterior circumference of the membranous septum and is located in part directly under the endocardium of the right ventricle: It proceeds along the interventricular septum and splits into many branches which spread along the entire internal surface of the right ventricle and along the papillary muscles of the trabeculae carneae and disappear in the substance of the myocardium.

The left branch is a wide, flat band which comes forward under the endocardium of the left ventricle in the upper portion of the interventricular septum, under the anterior edge of the posterior cusps of the aortic valve. It divides into two main branches at the border between the upper and middle thirds of the septum; then it separates, as in the right ventricle, into numerous, anastomosing thin threads which are lost to view in the myocardium.

The Blood Vessels of the Heart. The blood supply to the heart is carried by the coronary arteries. These are usually two in number and arise in the aortic sinuses. They are distributed to the capillaries of the myocardium. The blood from the capillaries is collected by the cardiac veins, most of which empty by way of the coronary sinus into the right atrium. A few very small cardiac veins empty directly into the right atrium.

In the coronary arteries of the human heart, the tunica media, which is limited on both sides by the usual internal and external elastic membranes, is divided by a thick fenestrated membrane into an inner and an external layer.

In ordinary preparations it is difficult to see blood vessels in the cardiac valves. By injec-

Fig. 225. Semidiagrammatic drawing based on several specimens, giving a composite picture of the typical vascular anatomy of a completely injected human mitral or tricuspid valve. Drawing by Max Brodel. 65 ×. After Bayne-Jones.

tion experiments Bayne-Jones found a rich, anastomosing net of vessels which extends to the line of closure in the atrioventricular valves (Fig. 225), but that in the semilunar valves the vessels extend only part way; he found no vessels in the center of the valve leaflets and none in the noduli Arantii. Others (Gross, Harper) hold that normal valves are practically devoid of vessels and that those which have been demonstrated are the result of chronic inflammatory processes (endocarditis).

There are a few vessels in the chordae tendineae; they run for the most part under the endothelium and arise from the vessels of the papillary muscles.

The sinoventricular system and, particularly, both of its nodes are abundantly supplied with

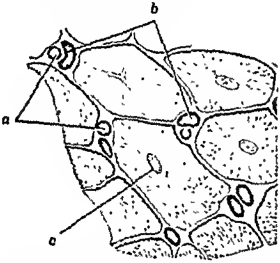

Fig. 226. Section of human myocardium, perpendicular to the muscle fibers, showing injected blood vascular capillaries, *a*, and lymphatic capillaries, *b*; *c*, nucleus of muscle fiber. High magnification Redrawn after Bock.

blood from special, rather constant branches of the coronary arteries.

Lymphatic Vessels of the Heart. Three groups of lymphatic vessels are described in the heart: (1) large lymphatic vessels which lie in the grooves of the heart together with the blood vessels; they are connected with the lymphatic nodes beneath the loop of the aorta and at the bifurcation of the trachea; (2) the lymphatic vessels of the epicardial connective tissue, and (3) lymphatic vessels of the myocardium and the endocardium.

In the subepicardial connective tissue ordinary flat networks of lymphatic capillaries may be demonstrated very easily These are connected with large efferent lymphatic capillaries and vessels.

Within the subendothelial connective tissue an even larger network of typical lymphatic capillaries exists; the larger vessels have valves. Lymphatic capillaries have been described in the atrioventricular and semilunar valves.

This lymphatic network in the endocardium was formerly often confused with the netlike ramifications of the sinoventricular system, for both structures may be demonstrated by the same injection method. But the conducting system forms much wider meshes and its cross bars are thicker and coarser.

The myocardium is penetrated by an abundant lymphatic network, which is everywhere connected with the subendocardial one, and also continues into the pericardial network. Each muscular fiber is surrounded by several lymphatic capillaries longitudinally oriented along its surface; these are connected by means of cross and tangential anastomoses and closely adjoin the blood vessel capillaries, which follow approximately the same direction.

The Nerves of the Heart. The numerous nerves of the heart belong in part to the vagus nerve and in part to the sympathetic nerves. For a detailed description see Kuntz, 1929.

Some nerve endings in the heart are apparently of effector type, while other endings are of receptor or sensory character. The nerve endings in the myocardium have been described (p. 197). Nonidez has given detailed descriptions of the nerve endings in the large arteries, near the heart, which are affected by changes in pressure in these vessels.

The Carotid and Aortic Bodies. These structures have until recently been erroneously included with the paraganglia. They do not contain chromaffin cells and have not been shown to have an internal secretion. The carotid and aortic bodies are similar in structure and presumably in function.

The carotid bodies are flattened, inconspicuous structures at the bifurcation of each common carotid artery. They contain irregular masses of pale-staining epithelial-like cells with pale nuclei closely applied to the endothelium of sinuses. The epithelioid cells are richly supplied with nerve endings apparently specialized to receive chemical stimuli (hence the name chemoceptors) indicating a fall in pH, a rise in carbon dioxide and a decrease in oxygen of the circulating blood. Hollinshead found a degranulation of the epithelioid cells when the oxygen tension was reduced to lethal levels. He believes the granules "are directly concerned with the initiation of chemoreceptor reflexes " The specific nerves from the carotid body reach the central nervous sys-

tem by the sinus branch of the glossopharyngeal nerve

The aortic body on the right side lies between the angle of the subclavian and the carotid, while on the left it is found above the aorta mesial to the origin of the subclavian, in each case occurring where the aortic nerve reaches the externa of the artery on which it ends (Nonidez). The structure of these bodies is identical with that of the carotid bodies.

The carotid body arises from the mesenchyme of the third branchial cleft artery and from the glossopharyngeal nerve. It is believed that the aortic bodies have a similar origin from the fourth branchial cleft artery and the vagus nerve.

The impulses from the aortic bodies are carried by the aortic nerve (depressor nerve of the vagus).

Chromaffin Cells. In the connective tissue between the aorta and pulmonary artery, approximately at the level of the semilunar valves, and also within the subepicardial connective tissue in the sulcus coronarius, mainly along the left coronary artery, small islands of chromaffin cells, *similar to the elements of the medullary substance of the suprarenal glands* are scattered (p. 313). They are in close connection with nerve networks and ganglion cells. In the newborn they are more highly developed than in adults.

The Histogenesis of the Blood Vessels and of the Heart. *Blood Vessels* The blood vessels and the heart first appear as a layer of endothelial cells In mammals the first vessels are laid down in the area vasculosa, where they develop from the mesenchymal cells (p 100) In the organism proper the blood vessels and the heart appear later; at first they are devoid of blood cells and are empty

In the spaces between the germ layers, groups of mesenchymal cells flatten around spaces filled with fluid which are thus surrounded by a thin endothelial wall In this way, in given places in the body, the primordia of the heart and the main blood vessels, as the aorta, cardinal and umbilical veins, etc, are laid down; then, these at first independent primordia rapidly unite with one another and with the vessels of the area vasculosa, after which the blood circulation is established. The endothelial cells in these first stages are merely mesenchymal cells adjusted to the new and special function of bounding the blood vessel lumen. The idea that the vascular system in the embryo proper arises as an ingrowth of vessels from the area vasculosa has been rejected by most observers. (See articles by McClure, Sabin, Clark.)

After the closed blood vascular system has developed and the circulation begun, new blood vessels always arise by "budding" from preexisting blood vessels.

The new formation of blood vessels by budding may be studied in sections of young embryos or in the living condition in the margin of the tail in larval amphibians, the mesentery of newborn mammals, or the thin layer of inflamed tissue which grows in between two cover slips introduced under the skin of an animal (Fig. 228). A method has been devised for the continued observation of such chambers in the living rabbit for weeks and even months (Clark, Sandison).

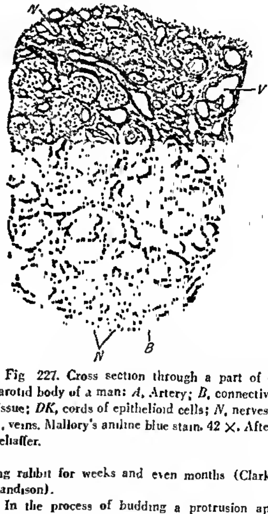

Fig 227. Cross section through a part of a carotid body of a man: *A*, Artery; *B*, connective tissue; *DK*, cords of epithelioid cells; *N*, nerves; *V*, veins. Mallory's aniline blue stain. 42 ×. After Schaffer.

In the process of budding a protrusion appears on the wall of the capillary and is directed into the surrounding tissues. From the beginning it often appears to be a simple, hollow expansion of the endothelial wall; in other cases it is at first a solid accumulation of endothelial cytoplasm. This *vascular bud* or sprout enlarges, elongates, and assumes many shapes. Most frequently it appears as a pointed cylinder. It always becomes hollow and thus represents a local outpouching of the blood vessel into which blood cells penetrate.

An endothelial bud may encounter another bud

and fuse with its end, or its lateral wall may come in contact with another bud or with another capillary. A lumen appears within the fused endothelial protoplasm and unites the two capillaries. In this way a new mesh is formed in the capillary network and blood begins to circulate in it. Later, new buds may arise from the newly formed vessels.

Arteries and veins of all types are always laid down at first as ordinary capillaries. The primary endothelial tube expands and thickens as new elements, uniting *with the outside of the* wall, differentiate in several directions. These elements originate from the surrounding mesenchyme in the embryo and form cells with mesenchymal potencies along the capillaries in the

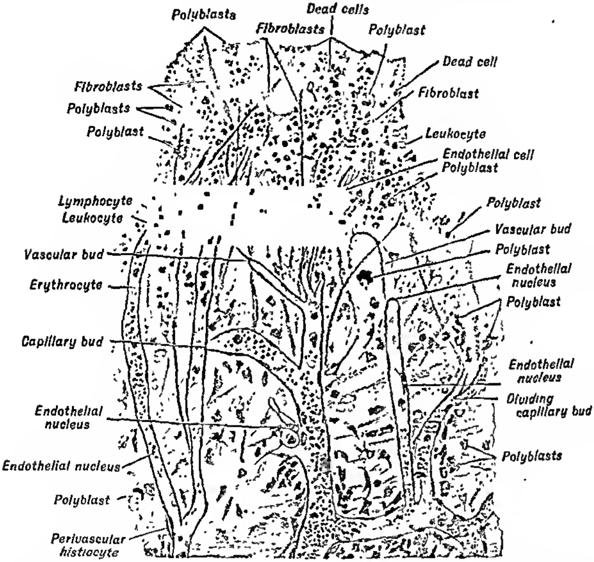

Fig. 228. Young connective tissue, with budding vessels, growing from below upward (in the figure) into the space between two cover slips inserted twenty days previously into the subcutaneous connective tissue of a rabbit. (A.A.M.)

The developing vascular buds are often accompanied by undifferentiated cells, phagocytes and fibroblasts, *stretched parallel to the long axis* of the buds; sometimes there are also wandering cells.

The most plausible explanation for the cause of the capillary bud is that the increase of metabolism within the tissue causes an increase in circulation of substances through the endothelium *and thereby* induces the growth of the endothelium in the direction of this current

adult. They play an important part in the new formation of arteries and veins from capillaries, *as well as in the formation of large vessels* from smaller ones in the development of a "collateral circulation" of the blood. The mesenchymal cells outside of the endothelium become young smooth muscle cells and myofibrils differentiate in their cytoplasm. Soon more layers of smooth muscle fibers join the first layer; these arise in part by multiplication of the existing smooth muscle cells and in part by the addition of new mesen-

chymal cells. In addition, networks of reticular fibers appear and form sheaths around the smooth muscle cells.

The factors which cause the larger arteries and veins to develop into more or less constant shapes in definite places and in definite directions are not completely solved. It is probable that in the earliest embryonic stages the formation of the vessels takes place through forces of heredity, while in the later stages the shape and the growth of the blood vessels are determined by local stimuli of mechanical and chemical nature.

The Heart. The heart at the beginning of the circulation is a tube with a double wall: the internal, endothelial layer from which the endocardium develops, and the external, myoepicardial. The latter consists of several layers of cells with indistinctly outlined boundaries. In the beginning (human embryo of 3 mm length) the distance between the two layers of the wall is rather great and is filled with a gelatinous, intercellular substance, which is penetrated by long, protoplasmic processes passing from the endothelium to the myo-epicardial layer.

In a human embryo 3.5 mm. in length, beginning with the sinus venosus and passing over to the atrium and the ventricle, this mucoid tissue disappears and the endothelium closely adjoins the myocardial layer. But in the vicinity of the opening which connects the atrium with the ventricle and in the bulbus this tissue remains. In this way, cushion like thickenings of the endocardium are formed; they consist of a mucoid connective tissue. The myocardium differentiates at the same time into an external peripheral layer of flat cells, the primordium of the serous membrane of the epicardium, and into the internal, thicker layer of irregular cells united into a syncytium by intercellular bridges. The histologic differentiation of the developing cardiac muscle from the syncytial layer located between the endo- and epicardium has been described in the section on the Cardiac Muscular Tissue.

The endocardial, cushion-like thickenings play an important rôle in the formation of partitions which separate the primary single cavity of the heart into compartments, and are particularly important in the formation of valves.

In the earlier stages of development the myocardium is continuous from the atria to the ventricles. But later, from the epicardium, along the course of the atrioventricular ridge, a transverse band of embryonic connective tissue develops which completely encircles the heart. It cuts into the myocardium from the exterior and separates entirely the muscle of the atria from that of the ventricles, save for the connection between them due to the atypical fibers of the conduction system.

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and fuse with its end, or its lateral wall may come in contact with another bud or with another capillary. A lumen appears within the fused endothelial protoplasm and unites the two capillaries. In this way a new mesh is formed in the capillary network and blood begins to circulate in it. Later, new buds may arise from the newly formed vessels.

Arteries and veins of all types are always laid down at first as ordinary capillaries. The primary endothelial tube expands and thickens as new elements, uniting with the outside of the wall, differentiate in several directions. These elements originate from the surrounding mesenchyme in the embryo and form cells with mesenchymal potencies along the capillaries in the

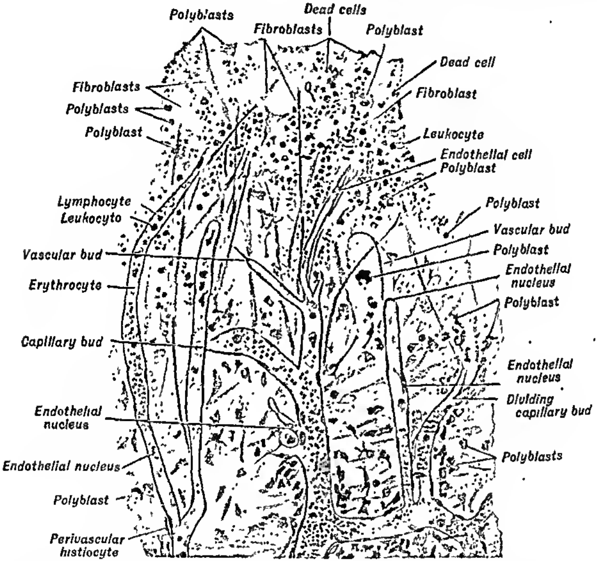

Fig. 228. Young connective tissue, with budding vessels, growing from below upward (in the figure) into the space between two cover slips inserted twenty days previously into the subcutaneous connective tissue of a rabbit (A.A.M.)

The developing vascular buds are often accompanied by undifferentiated cells, phagocytes and fibroblasts, stretched parallel to the long axis of the buds; sometimes there are also wandering cells.

The most plausible explanation for the cause of the capillary bud is that the increase of metabolism within the tissue causes an increase in circulation of substances through the endothelium and thereby induces the growth of the endothelium in the direction of this current.

adult. They play an important part in the new formation of arteries and veins from capillaries, as well as in the formation of large vessels from smaller ones in the development of a "collateral circulation" of the blood. The mesenchymal cells outside of the endothelium become young smooth muscle cells and myofibrils differentiate in their cytoplasm. Soon more layers of smooth muscle fibers join the first layer; these arise in part by multiplication of the existing smooth muscle cells and in part by the addition of new mesen-

Chapter XI

THE LYMPHATIC SYSTEM

An exchange of nutritive materials and oxygen proceeds continuously between the blood within the capillaries and the tissue juice bathing the cells of the various tissues. Most of the waste products of metabolism are returned from the tissues to the capillaries and capillary veins. In the vertebrates the vessels of the closed lymphatic system return some of the tissue fluids to the general circulation by a roundabout route.

The lymphatic system is composed of *lymphatic vessels and organs.* The smallest vessels, the *lymphatic capillaries,* are thin-walled, blindly-ending tubes which form a dense network in most of the tissues of the body. They collect tissue juice which is called lymph as soon as it enters these capillaries. The lymphatic capillaries unite to form larger vessels, the largest of which *empty into veins.* The lymphatic system thus differs from the blood vascular system in that it is not a closed vascular ring. The lymphatic organs are located along the course of the lymphatic vessels and contribute various sized lymphocytes to the lymph passing through them. The lymph of the finest lymphatic radicles is almost devoid of cells.

Certain cavities in the body are more or less directly connected with the lymphatic system. In this group are the serous cavities (peritoneum, pleura, pericardium), the spaces surrounding the meninges, the chambers of the eye, Tenon's cavity around the eyeball, the cavity of the internal ear filled with endolymph, the ventricles of the brain, and the central canal of the spinal cord. The liquids in these cavities are quite different from the lymph and have quite a different physiologic significance, although the fluid in the serous cavities is much like lymph. Nevertheless, injected colloidal solutions and particulate matter may penetrate from these cavities into the true lymphatic vessels and vice versa. The slitlike cavities and spaces in the connective tissue are not lymphatic spaces but are called tissue spaces.

LYMPHATIC CAPILLARIES AND VESSELS

Lymphatic capillaries are thin-walled, tubular structures of slightly greater caliber than blood capillaries. Unlike the latter, which usually have a regular cylindrical form, they have irregular shapes and are constricted in some places and dilated in others. They branch abundantly and anastomose freely with one another. Dilatations occur frequently where several capillaries join. The lymphatic networks are often located beside networks of blood capillaries, but are always independent of them. As a general rule, the lymphatic networks are farther from the surface of the skin or mucous membranes than the blood capillary networks.

Further, the lymphatic networks are distinguished from the blood capillaries by ending blindly in rounded or swollen ends. This is best seen in the mucous membrane of the small intestine where a network of lymphatic capillaries or a single, blindly-ending vessel, the *central lacteal,* extends in the lamina propria up to the end of the villus (Fig. 229). The lymphatic capillaries form expanded networks of considerable size around the solitary and aggregated lymphatic nodules

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sists of interlacing collagenous and elastic fibers, and smooth muscle bundles. The elastic fibers of the tunica adventitia continue into those of the surrounding connective tissue.

Valves. The valves of the lymphatic vessels always occur in pairs; they are placed on opposite sides of the vessel and their free edges point in the direction of the lymph flow (Fig. 231). The valves are frequently unable to withstand the pressure of a retrograde injection. As in the veins, the valves of the lymphatic vessels are folds of the tunica intima. They have a thin connective tissue base and are covered on both sides by a layer of endothelium continuous with that of the rest of the vessel. Although valves are not present in all lymphatic vessels, when they occur they are usually much closer together than those in the veins.

Above each pair of valves, the lymphatic vessel is more or less distinctly expanded and the wall in these places has several prominent layers of smooth muscles in its media. It is believed by some that the contractions of these muscles may help move the lymph along the vessel. Because of these swellings the shape of a distended lymphatic vessel which contains many valves may suggest a rosary.

Large Lymphatic Vessels. Thoracic Duct. The lymphatic vessels unite with other similar vessels and become larger and larger, while their walls become thicker. They form more or less complicated networks which often surround blood vessels. This is very marked about the mesenteric vessels of some mammals.

Finally, all of the lymphatics come together and form two main trunks—the right lymphatic duct and the thoracic duct. The former is the smaller; it carries the lymph from the upper right portion of the body and usually opens into the right innominate vein where it arises from the right internal jugular and subclavian veins. The thoracic duct carries the lymph from all of the remaining parts of the body (including the digestive system) and opens into the point of junction of the left internal jugular and subclavian veins. Both of the ducts are provided with valves where they enter the veins.

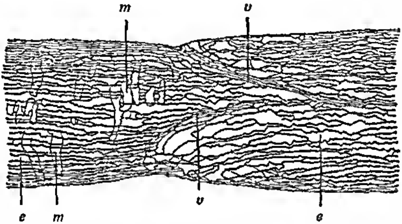

Fig. 231. Small lymphatic vessel of the mesentery of a rabbit The outlines of the endothelial cells, *e*, are stained black with silver nitrate; *v*, lines of attachment of the valves; *m*, transverse smooth muscle cells. 140 ×. Redrawn after Kolliker v. Ebner.

The wall of the thoracic duct differs from that of the great veins by the greater development of the muscles in the tunica media, by a less distinct division into three layers, and, particularly, by the great irregularity in the structure of adjacent portions.

The tunica intima consists of the endothelial lining and several thin layers of collagenous and elastic fibers; the latter condense into a layer similar to an internal elastic membrane near the junction with the tunica media. The transverse smooth muscle bundles in the tunica media are

of the intestine, and in the thyroid and mammary glands.

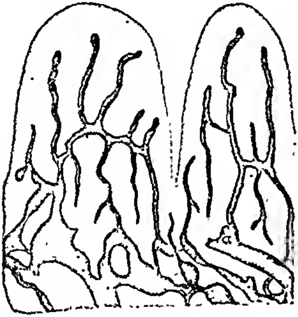

Fig. 229. Lymphatic capillaries (lacteals) filled with Berlin blue in the villi of the intestine of a rat. Redrawn after Ranvier.

The wall of the lymphatic capillaries is formed by a single layer of flat endothelial cells; these are slightly larger and thinner than those of the blood capillaries Hence, in sections of collapsed lymphatic capillaries, only the endothelial nuclei can be seen and these cannot be distinguished from the nuclei of the surrounding fibroblasts. The general form of the endothelial cells is usually rounded or polygonal and only rarely elongated. Each cell contains an oval, flattened nucleus. The lymphatic capillaries abut directly against the surrounding tissues and are not provided with a layer of pericytes like the blood capillaries.

The lymph passes from these capillary networks into lymphatic vessels which have slightly thicker walls and valves. They are covered at first by thin, mainly longitudinal, collagenous bundles, elastic fibers, and a few smooth muscle cells, arranged tangentially or transversely to the vessel. Those lymphatic vessels with a diameter greater than 0.2 mm. have thicker walls in which three layers, corresponding to the inner, medial, and adventitial coats of arteries and veins, can be distinguished. The boundaries between these layers are often indistinct, so that the division is somewhat artificial. The tunica intima consists of the endothelium and a thin layer of longitudinal, interlacing elastic fibers. The tunica media is composed of several layers of mainly circular and a few tangential smooth muscles, and several thin elastic fibers. The tunica adventitia is the thickest layer and con-

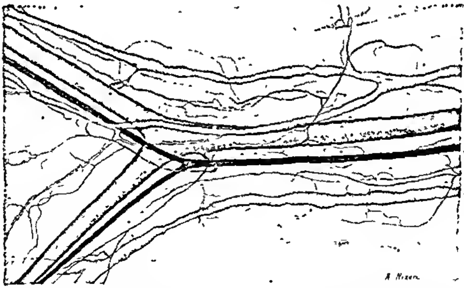

Fig. 230. Vital injection of lymphatic vessels with alpha-azurine F. G. and of the blood vessels with colloidal mercuric sulfide of the mesentery of a rabbit. About 20 ×.

or *sinuses*. The nodes are always located along the course of lymphatic vessels, whose contents pass through the nodes on their way to the thoracic and the right lymphatic ducts. Lymph nodes are scattered in large numbers, usually in groups, throughout the prevertebral region, in the mesentery, and in the loose connective tissue of the inner surfaces of joints, as the axilla, groin, etc. They are flat, well-defined bodies varying from 1 to 25 mm. in diameter. Their form is rounded or kidney-shaped and their surface is somewhat rough. Usually there is a slight indentation, the *hilus*, on one side of the node, where blood vessels enter and leave the organ. Lymphatic vessels enter the node at many places over its convex surface; they leave it only at the hilus.

The sectioned surface of a lymph node under low magnification shows the organ to be surrounded by a dense connective tissue *capsule* and to be divided into an outer *cortical* and an inner *medullary* part.

The cortex usually occupies the surface of the organ, with the exception of the hilus, and contains closely packed, roughly spherical, white areas about 1 mm. in diameter, the nodules. These are

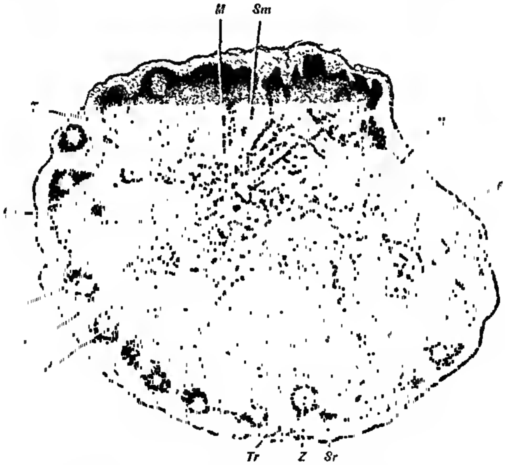

Fig. 233. Section through a small jugular lymph node of man. *Ca*, Capsule; *F*, nodules with their centers, *Z*; *f*, fat tissue; *h*, hilus; *M*, medullary cord; *Sm*, medullary and, *Sr*, subcapsular sinus; *Tr*, trabeculae; *v*, blood vessel. 18 ×. Redrawn and slightly modified from Sobotta.

embedded in a diffuse mass of lymphatic tissue which continues as the medullary cords into the medulla. The red-brown medulla is not sharply separated from the cortex and usually occupies the inner portion of the node radiating from the hilus. The differences in appearance between the cortex and medulla consist mainly in differences in arrangement of the elements of the lymphatic tissue in the two zones.

penetrated by elastic fibers coming from the elastica interna. The tunica adventitia is composed of longitudinal collagenous fibers, interlacing elastic fibers, and a few longitudinal smooth muscle bundles. The tunica adventitia gradually merges into the surrounding loose connective tissue.

Blood Vessels of Lymphatics. The wall of the thoracic duct is provided with many blood vessels which extend into the outer layer of the middle tunic; these vessels are similar to the vasa vasorum of the larger blood vessels. The narrow, thin-walled lymphatic vessels are often accompanied by a small artery and a vein which run parallel to it. Capillaries arise from them and encircle the lymphatic vessel or form regular networks on its surface.

Fig 232 Section of periportal area of a human liver. 480 ×.

Nerves of the Lymphatics. Both the large thoracic duct and the smaller lymphatic vessels are abundantly supplied with nerves. In both adventitial and medial coats some of the fibers terminate in sensory endings The other fibers are motor nerves for the smooth muscles, as in the blood vessels.

Passage of Lymph from the Tissues into the Lymphatics.

Investigation with the aid of injection methods has shown that the lumen of lymphatics does not communicate directly with the tissue spaces. The so-called "stomata" seen in silver nitrate preparations are undoubtedly artefacts. As the lymphatics form a closed, endothelium-lined system of tubes, the tissue juice must pass through the endothelial cytoplasm to reach the lumen of the lymphatics. In inflammation the permeability of the local lymphatics to certain dyes is increased (McMaster and Hudack, 1932).

LYMPHATIC ORGANS

Closely connected with the lymphatic vessels are collections of *lymphatic tissue* which are aggregated into the *lymphatic organs.* The lymphatic tissue has been discussed in Chapter V. The tonsils and the solitary and aggregate follicles of the intestine are described in the section on the digestive system and the spleen on page 270. Here only the lymph nodes will be considered.

LYMPH NODES

The lymph nodes are large accumulations of lymphatic tissue and consist essentially of networks of reticular and collagenous fibers which support masses of lymphocytes and are intimately connected with primitive reticular cells and many fixed macrophages The whole mass is penetrated by tortuous *lymphatic spaces*

or *sinuses*. The nodes are always located along the course of lymphatic vessels, whose contents pass through the nodes on their way to the thoracic and the right lymphatic ducts. Lymph nodes are scattered in large numbers, usually in groups, throughout the prevertebral region, in the mesentery, and in the loose connective tissue of the inner surfaces of joints, as the axilla, groin, etc. They are flat, well-

under low magnification shows the organ to be surrounded by a dense connective tissue *capsule* and to be divided into an outer *cortical* and an inner *medullary* part.

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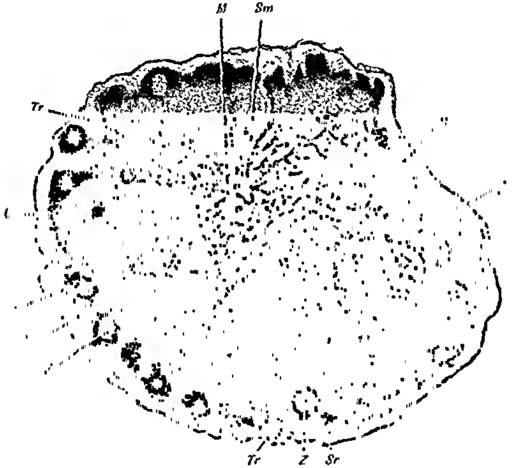

Fig. 233. Section through a small jugular lymph node of man. *Ca*, Capsule; *F*, nodules with their centers, *Z*; *f*, fat tissue, *h*, hilus; *M*, medullary cord; *Sm*, medullary and, *Sr*, subcapsular sinus; *Tr*, trabeculae; *v*, blood vessel. 18 ×. Redrawn and slightly modified from Sobotta.

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The sectioned surface of a lymph node

embedded in a diffuse mass of lymphatic tissue which continues as the medullary cords into the medulla. The red-brown medulla is not sharply separated from the cortex and usually occupies the inner portion of the node radiating from the hilus. The differences in appearance between the cortex and medulla consist mainly in differences in arrangement of the elements of the lymphatic tissue in the two zones.

The *afferent lymphatic vessels* lead into a broad lymphatic channel, the *subcapsular sinus,* whence the lymph passes by other broad or narrow passages to dilated sinuses between the medullary cords in the medulla of the organ. From here the lymph is conducted by the *efferent vessels* toward the thoracic duct.

The lymphatic tissue of the lymph nodes consist of dense accumulations of cells in the cortex (with its nodules) and in the medullary cords, and of looser areas forming the sinuses. Most of the free cells are lymphocytes of various sizes (p. 79). Plasma cells often occur, especially in the medullary cords of the mesenteric nodes of the rat. A few hematogenous eosinophil leukocytes can be found in most lymph nodes.

The cellular stroma is made up of dye-storing fixed macrophages and primitive reticular cells (which do not store vital dyes). The sinuses, particularly of the medulla, contain free macrophages, even under normal conditions. The reticular fibers of the framework are generally believed to be formed by the fixed macrophages or primitive reticular cells (or both) as typical fibroblasts are found only in the capsule and trabeculae.

Framework. The framework of the lymph nodes consists of the capsule, the trabeculae, and the network of reticular fibers.

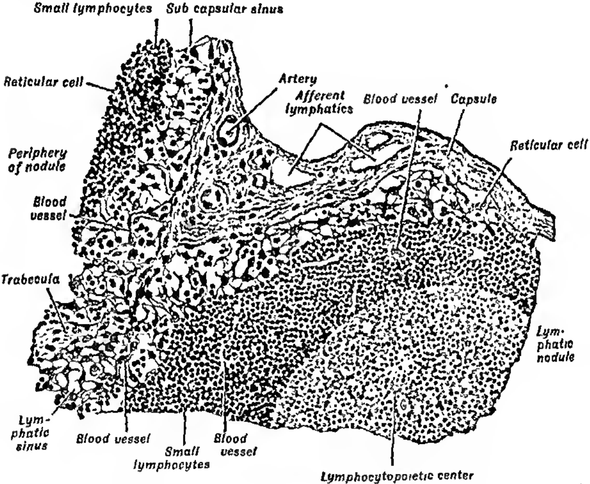

Fig. 234. Portion of cortex of a lymph node of a dog. Hematoxylin eosin azure stain. 187 ×. (A.A.M.)

Capsule. The periphery of the node is covered by the capsule, which consists of dense bundles of collagenous fibers, a few fibroblasts and, particularly on its inner surface, networks of thin elastic fibers. A few smooth muscle cells are also found in the capsule about the points of entry and exit of the afferent and efferent lymphatic

vessels. At the hilus the capsule is greatly thickened and may extend for some distance into the medullary portion of the node.

Trabeculae. At various points along the periphery of the node, projections of dense connective tissue, the trabeculae, arise from the capsule and pass into the medulla of the node, branch into a number of shafts which finally fuse with the collagenous tissue of the capsule at the hilus. The collagenous fibers of the trabeculae are frequently continuous with the reticular fibers.

Reticular Fibers. The reticular fibers penetrate all parts of the node and form

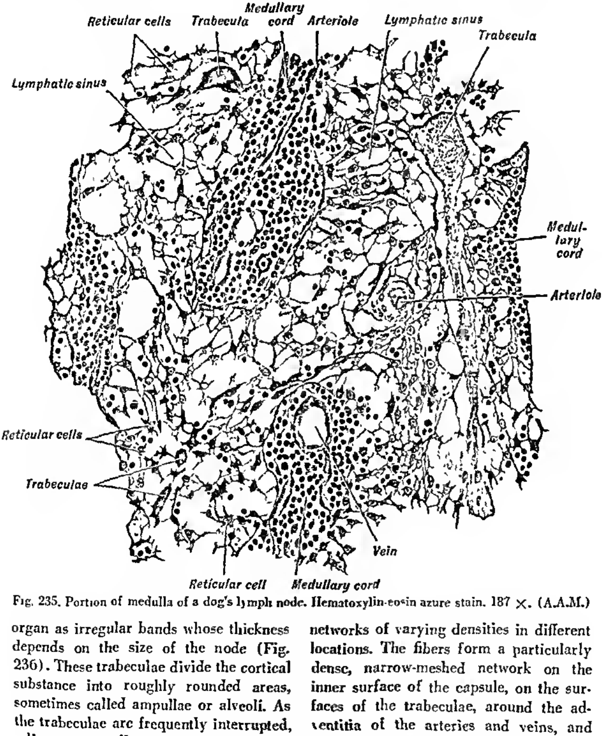

Fig. 235. Portion of medulla of a dog's lymph node. Hematoxylin-eosin azure stain. 187 ×. (A.A.M.)

organ as irregular bands whose thickness depends on the size of the node (Fig. 236). These trabeculae divide the cortical substance into roughly rounded areas, sometimes called ampullae or alveoli. As the trabeculae are frequently interrupted, adjacent ampullae connect with each other. The trabeculae, on reaching the networks of varying densities in different locations. The fibers form a particularly dense, narrow-meshed network on the inner surface of the capsule, on the surfaces of the trabeculae, around the adventitia of the arteries and veins, and throughout the dense lymphatic tissue. Between these areas (with dense reticular

fiber networks) there are looser networks of reticular fibers through whose meshes lymph percolates. These loosely meshed areas constitute the sinuses. In the center of the nodules the reticular fibers are very thin and scarce or may be absent.

The Cortical Substance. At the periphery of each ampulla, *i. e.*, under the capsule and bordering the trabeculae, the cortical substance is very loose and usually consists of a fairly wide lymphatic channel or sinus (Fig. 234) (see below).

The nodules are temporary structures, expressing the cytogenic and defense functions of the lymphatic tissue, which depend on the age, condition or nutrition of the organism, etc. They may develop and may disappear, to reappear again at the same or another place. The number and size of the nodules fluctuate remarkably. In the embryo and in the first months after birth they lack the central "germinal" or "reactive" areas. With the growth and development of the organism these centers appear and then become more numerous and larger. With advancing age they become less conspicuous and smaller, and in old age and in various diseases may disappear.

The Medullary Substance. The medullary substance consists of the same cytological constituents as the cortex, although the elements are arranged differently. The *medullary cords* are dense lymphatic tissue and rarely contain nodules. The cords branch and anastomose freely with one another. Near the hilus they terminate with free ends or, more frequently, they form loops which continue into other cords. The cords are accompanied and surrounded by the *medullary sinuses* which separate them from the trabeculae and are continuations and amplifications of the cortical sinuses. The substance of the sinuses is also composed of lymphatic tissue, but its meshes are so wide that they constitute relatively broad channels for the passage of lymph.

Lymphatic Vessels and Sinuses. The vessels which supply lymph to the node (vasa afferentia) are provided with valves which open toward the node (Fig. 236, *a*). These afferent vessels approach the convex surface of the node, pierce its capsule and open into the subcapsular sinus. From here the lymph passes through the looser parts of the lymphatic tissue, the sinuses, of both the cortex and medulla, and then into the efferent lymphatic vessels at the hilus (Fig. 236, *e*).

Unlike the tubular, endothelium-lined blood vascular and lymphatic *vessels*, the lymphatic *sinuses* are irregular, tortuous spaces within the lymphatic tissue. Their walls are not continuous and are formed of reticular cells and fixed macrophages supported by the reticular fibers. As a continuous stream of lymph flows through the sinuses, lymphocytes are swept into the efferent lymphatic vessels, and new lymphocytes enter the sinuses by their own ameboid movement.

The sinuses of the medullary substance at first pass over into a network of twisted tubes, which penetrate the thickened portion of the capsule at the hilus and then continue into the efferent vessels which lead the lymph away. These are wider and less numerous than the afferent vessels; they are provided with valves which open away from the node. The arrangement of the valves in the afferent and efferent vessels thus permits a flow of lymph in only one direction through the node.

The margins of the endothelial cells in the lymphatic vessels can be outlined by treatment with silver nitrate. The outlines of the reticular cells which form the walls of the lymphatic sinuses may sometimes be demonstrated by this means when they are so closely packed as to simulate endothelial cells. In all cases their reticular nature is easily recognizable.

Variations in Structure of Lymph Nodes. The above-described arrangement of the constituents of a typical lymphatic node is realized in but few instances, for the lymph nodes show

great variations in structure depending on the animal species as well as the location of the node. But none of these deviations affects the fundamental structure.

In large nodes the trabeculae are prominent, in small nodes they are very thin and are frequently interrupted so that they may be absent for long stretches. The nodes which are deep in the body, as those in the peritoneal cavity, are also distinguished by the poor development of their trabeculae as contrasted with the more peripheral nodes.

In some cases, a hilus may be absent, while in others it may be so highly developed that its connective tissue may penetrate far into the node and divide it completely. In the ox the trabecular system is so well developed that the nodules of the cortex are completely separated from one another. When the trabecular system is poorly developed, as in man, the nodules of the cortical substance and the sinuses may lose their sharp outlines and often fuse into a continuous, diffuse mass of lymphatic tissue, in which there are occasional looser strips or passages along which the lymph flows. Such areas when filled with macrophages have been called "interfollicular tissue." The term should be discarded—as the only tissue in the node is lymphatic tissue.

The relative amounts of cortical and medullary substance and their mutual arrangement fluctuate within very wide limits. The nodes of the abdominal cavity are especially rich in medullary substance. In those cases where the cortical substance predominates, the nodules may be arranged in several layers. Sometimes, the cortical substance may surround the medulla completely while in other cases the medullary substance may be adjacent to the capsule for long distances. In some cases the medulla and cortex may accumulate at opposite poles of the node, while in the pig the cortical substance with its nodules is collected in the central portion of the node and the medullary cords with their wide sinuses may occupy only small portions of the periphery.

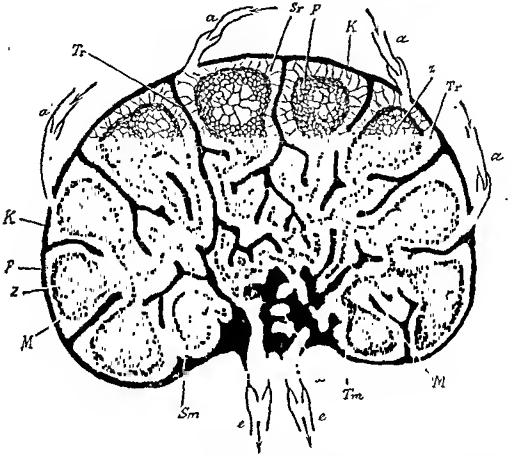

Fig. 236 Diagram of a lymph node. *a*, Afferent and, *e*, efferent lymphatic vessels with valves; the arrows indicate the direction of lymph flow; *F*, cortical tissue; *K*, capsule; *M*, medullary cords; *Sm*, medullary and, *Sr*, cortical sinuses; *Tm*, medullary trabeculae which are continuous with those of the cortex; *Tr*, trabeculae originating in the capsule and dividing the cortex into ampullae; *x*, lymphatic vessels in the dense connective tissue of the hilum, *h*; *Z*, nodules.

Blood Vessels. Almost all the blood vessels

destined for the lymph node enter it through the hilus and only occasionally small ones through the capsule. The larger arterial, as well as venous branches, pass along the trabeculae, while the smaller ones pass along the axis of the medullary cords toward the cortex. The capillaries into which the arteries split in all parts of the lymphatic tissue form particularly dense networks in the peripheral layers of the medullary cords and of the nodules. In the latter they form radially arranged meshes. The blood capillaries of lymphatic tissue, particularly in the cortex, have a thickened endothelium, so that in cross section they often appear as though lined by cuboidal epithelium. Here, large numbers of newly formed, small lymphocytes are present and pass through this endothelium from the tissue directly into the blood.

Nerves. The nerves enter the hilus of the node with the blood vessels which they follow, forming perivascular networks. In some places in the trabeculae and in the medullary cords, independent nervous networks may be noticed, but in the nodules the nerves are present only along the course of the vessels. They are probably of the vasomotor type.

Hemal Nodes. Even in normal lymph nodes varying numbers of erythrocytes are found; these have either entered the lymph from the afferent vessels or have come from the blood vessels of the node. Some of these erythrocytes are eliminated with the lymph into the efferent vessels, but most of them are engulfed by the fixed macrophages. There are some nodes, however, which are characterized by their great content of erythrocytes; macroscopically, such organs are called *hemal nodes;* they are most numerous and well defined in the ruminants (sheep); they probably do not occur in man.

These oval or spherical organs vary from the size of a hardly noticeable granule to that of a pea or larger, and are scattered in the vicinity of large blood vessels in the retropleural and retroperitoneal tissues along the vertebral column from the neck to the pelvic inlet. They are also found near the kidneys and spleen where they are believed by some to be accessory spleens.

Each node is covered by a dense capsule loosely connected with the surrounding tissue. At the hilus a small artery and a large vein enter and leave. The hemal nodes are devoid of afferent and efferent lymphatics.

The hemal node is composed of a more or less continuous mass of lymphatic tissue, separated from the capsule by a sinus filled with blood. The lymphatic tissue frequently projects into the peripheral sinus by a number of follicle-like outgrowths and is, in turn, penetrated by the more or less distinct "interstitial" sinuses originating from the peripheral sinus. These may continue into the "central" sinuses in the deeper portions of the node. All of the sinuses are filled with blood. A direct connection between the blood-containing sinuses and branches of arteries or veins has not as yet been demonstrated.

The hemal nodes may be considered as "filters" of lymphatic tissue, situated in the course of blood vessels, and the principle of their structure is closer to that of the spleen than of ordinary lymph nodes. In the pig a special type of hemolymphatic node occupies a position halfway between the ordinary lymph node and the typical hemal node. It has blood as well as lymphatic vessels and the contents of both types of vessels mix in the sinuses. It is possible that even in adult animals a simple lymph node may change into a hemal node and vice versa.

The functions of the hemal nodes are probably like those of the spleen.

Function of Lymphatic Nodes. Although they share this function with all the other accumulations of lymphatic tissue in the body, they are the most active structures for the formation of the lymphocytes. The stimuli for lymphocyte production are probably brought to the lymph nodes by both lymphatic and arterial vessels. Although great numbers of lymphocytes are produced in certain infections, the lymphatic leukemias, and some intoxications—as diphtheria—the actual stimuli for lymphocytopoiesis in these conditions, as well as in physiological states, are unknown. As the lymph nodes are composed essentially of lymphocytes and phagocytes, it is obvious that their main functions depend on these cells. The functions of the lymphocytes are discussed in Chapter V and those of the macrophages on pages 62, 96 and 114.

In some pathologic conditions, extramedullary myelopoiesis occurs and the nodes become the site of formation of granular leukocytes (p. 112).

Because of the phagocytic activity of the reticular cells, particularly in the sinuses, the nodes serve as filters in which various particles, arising locally or

brought with the lymph from other regions of the body, are taken up and often destroyed. Even in normal conditions, erythrophagocytosis can be seen in the sinuses of lymph nodes. This process is much more prominent when great numbers of erythrocytes are brought to the nodes as a result of hemorrhage into the nearby tissues. Particles of coal dust which are inhaled by the lungs finally enter the bronchial lymph nodes where they are taken up by the reticular cells and often accumulate in such quantities that the organ becomes black. Pathogenic bacteria brought to the lymph nodes are frequently ingested and sometimes destroyed by the macrophages. Just like all the other tissues and organs containing many macrophages, the lymph nodes probably elaborate antibodies. It is possible that lymphocytes contain antibodies (see p. 84).

Histogenetic Remarks. In the mammalian embryo the lymphatic system is laid down much later than the blood vascular system. The lymphatic vessels arise first and the lymphatic organs develop in connection with them somewhat later.

Lymphatic Vessels. Although there are many unsettled details in the question of the mode of development of the lymphatic system, most observers believe that the primordia of the lymphatic sacs and vessels arise independently of the veins, although often close to them, as isolated small clefts in the mesenchyme, which are filled with tissue fluid and surrounded by mesenchymal cells. The latter, owing to the pressure exerted by the fluid, acquire a flattened appearance and the character of endothelium. These spaces gradually fuse, forming in certain places large cavities, the *lymphatic sacs*, as well as vessels of more or less cylindrical shape The sacs later communicate with the adjacent veins.

The vessels elongate rapidly in all directions due to a continued addition of new cavities arising in the mesenchyme. The presence of blood in the early lymphatic vessels is explained as being due in part to a flow of blood from the veins and in part to the appearance of local hemopoietic islands in the mesenchyme together with the lymph sacs. These blood cells become included in the latter and are carried with the lymph into the veins.

After a certain stage the further development of the lymphatic system takes place mainly by budding of the endothelium of existing lymphatic vessels. These outgrowths may be observed directly in the tail of living amphibian larvae and in chambers in the rabbit's ear. They agree very closely with the outgrowths from blood vessels (p. 253).

As in the blood vascular system, the developing lymphatic system does not retain all of the parts laid down in the beginning; its constituents continue to change and become reconstructed. The main parts of the primary lymphatic system, the *sacs*, spread irregularly in various directions, and change their form; they develop in part into networks of lymphatic vessels and in part into complexes of lymphatic nodes (see below).

The student is referred to Zimmermann's monograph for the latest survey of the field.

Valves. Valves appear in the lymphatic vessels several weeks before they develop in the blood vessels. They appear first in the lymphatics near the jugular sacs and the upper part of the thoracic duct, then in the lymph vessels of the appendages, and finally in the remainder of the thoracic duct. They consist, as do those of the veins, of a connective tissue base and a covering of endothelium.

Lymphatic Nodes. The development of the lymph nodes begins after the formation of the primary lymphatic vascular system. The earliest or primary nodes develop by a transformation of the lymphatic sacs. Each sac disappears as such and separates into a group of connected networks of lymphatic vessels which become nodes of various sizes; portions of the primary cisterna chyli and of the jugular sacs remain as cavities. As the sacs are at first the centers of development of the lymphatic vessels in a given region of the body, so in the future all the lymph collected from that region is finally carried into the corresponding group of deep primary nodes, as the deep jugular nodes, retroperitoneal, etc. The secondary nodes, such as the peripheral, the inguinal, etc., appear later along the course of lymphatic vessels; many smaller nodes are apparently formed after birth.

The transformation into a primary node is carried out by an invagination, into the lumen of the sac, of the surrounding mesenchyme, which grows through the sac in thick or thin bars. The mesenchyme forming the bars or partitions between the cavities at first does not contain wandering cells.

According to the newer investigations, the lymphatic sinuses arise as irregular, blind, and anastomosing spaces, which are lined from the beginning by flattened mesenchymal cells, and

only later do they come in contact with the endothelium-lined afferent and efferent vessels.

The primary node is a common primordium for the lymphatic tissue of the cortex and medulla, *from which the medullary substance arises first.*

The lymphocytes develop *in situ* by the isolation and rounding up of mesenchymal elements and later through their own multiplication. They accumulate mainly in the marginal sinus and are carried away by the lymph stream. Among

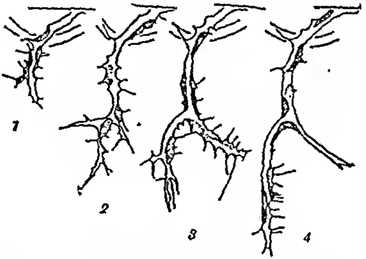

Fig. 237. Successive stages (1–4) of the growth of a bud of a lymphatic capillary of a frog tadpole during three days. 180 ×. Redrawn after E. R. Clark.

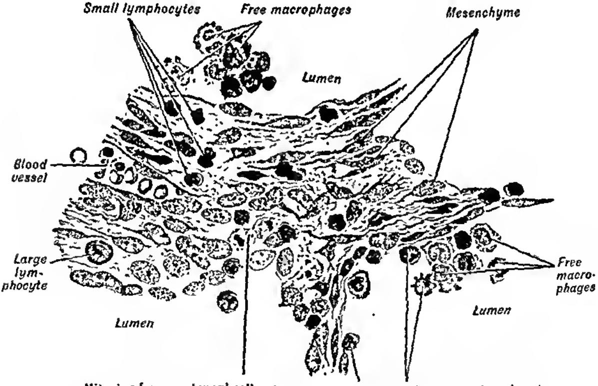

Fig. 238 Primordium of a lymph node in the wall of the cervical lymph sac of a human embryo of 37 mm. Lumen of the sac is divided into cavernous spaces by partitions of mesenchyme. Eosin-azure stain. About 400 ×. (A.A.M.)

The true cortical substance appears much later as the medullary cords on the periphery of the node gradually develop club-shaped thickenings which bulge into the marginal sinus. The development of the lymphatic nodules is completed very late, in the majority of cases after birth.

these cells, at first the small ones always predominate, but large lymphocytes and macrophages also occur. Granulocytes and megakaryocytes appear temporarily with the lymphocytes, but soon disappear. The mesenchymal elements, which did not transform into lymphoid cells,

either remain as undifferentiated elements (primitive reticular cells) or give rise to the fixed macrophages of the stroma (see p. 78). Fibrils appear rather late in the stroma of lymphatic tissue.

Hemal Nodes. These are first laid down on the same plan as the ordinary nodes; a central mass can be distinguished in them, which transforms into lymphatic tissue and is surrounded by the marginal lymphatic network or sinus. Blood enters the latter quite early and is brought here in part by the lymphatic vessels and in part comes from the adjacent tissue areas where hemorrhages occur due to degeneration of blood vessels. Then the marginal sinus with the efferent and afferent lymphatic vessels is lost.

Regenerative Capacity of the Lymphatic System. When the adult human body is incised or otherwise injured, lymphatic vessels, and sometimes lymphatic organs, are injured. Regeneration of the vessels begins in the lymphatic capillaries and proceeds by vascular budding. In some cases, however, for reasons which are not known, regeneration of the lymphatic vessels does not take place.

The *tissue of the lymphatic nodes responds to* local injury at first by the rounding up of reticular cells and their transformation into macrophages which multiply by mitosis. The lymphocytes, which at first are unchanged, then begin to multiply and hypertrophy into polyblasts. But this attempt at regeneration is limited, and healing is usually brought about by the development of ordinary scar tissue.

After excision in young rabbits, lymph nodes may regenerate from local cells. With advancing age the regenerative ability decreases markedly.

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Chapter XII

THE SPLEEN

The spleen is one of the blood-forming and destroying organs and plays important rôles in the metabolism and defense mechanisms of the body. It is the largest mass of lymphatic tissue in the body. But unlike the other collections of this tissue which are interposed in the lymph stream, the spleen is inserted in the blood stream. Owing to a peculiar type of blood vessel which allows the circulating blood to come into very close contact with the macrophages of this organ, the spleen acts in many respects as a filter for the blood; this property becomes greatly accentuated in immune reactions.

The spleen is covered by a thin, elastic *capsule* which adheres to the underlying tissue. The capsule is thickened at the hilus of the organ where it is attached to folds of the peritoneum and where arteries enter and veins leave the viscus. Branching and anastomosing continuations of the capsule, called *trabeculae,* penetrate the organ and form part of its framework.

The splenic tissue which fills the spaces between the trabeculae is composed of typical lymphatic tissue (*white pulp*) and an atypical lymphatic tissue—the *red pulp.* The red pulp is a pastelike, dark red mass which can be scraped from the cut surface of the organ. On a freshly sectioned surface of the spleen the white pulp is seen as irregular long or rounded gray areas, 0.2 to 0.7 mm. in diameter, scattered throughout the red pulp. These white areas are often called Malpighian bodies after the anatomist who first described them. They consist of diffuse and nodular lymphatic tissue, which varies considerably in its finer structure from time to time. It is inadvisable to use the term Malpighian body, as it has been interpreted to mean different structures by various histologists.

The structure of the spleen and the relations between the red and white pulp depend on the distribution of the blood vessels. The arteries are closely connected with the white pulp and the veins with the red pulp. There is sharp disagreement between various investigators as to the mode of connection between the arteries and veins.

The Capsule and Trabeculae. The capsule and the trabeculae of the spleen consist of dense connective tissue and a few smooth muscle cells. The collagenous fibers of the trabeculae are continuous with the reticular fibers of the pulp. Elastic fibers form a network between the collagenous bundles. In man, the network of the thickest elastic fibers is located in the deep layers of the capsule. The external surface of the capsule is covered by a layer of flattened mesothelium which is part of the peritoneum.

In the trabeculae the elastic fibers are more numerous than in the capsule and sometimes replace most of the collagenous fibers. *Muscle fibers* are present in small groups (in man) or in long cords. The slow rhythmical changes in the volume of the organ are due to the smooth muscle in the capsule and trabeculae (in those species in which smooth muscle is

prominent) and to the vascularly controlled changes in the amount of blood in the organ.

lar cells or fixed macrophages (p. 78). As in all lymphatic tissue the meshes of the framework are filled with free

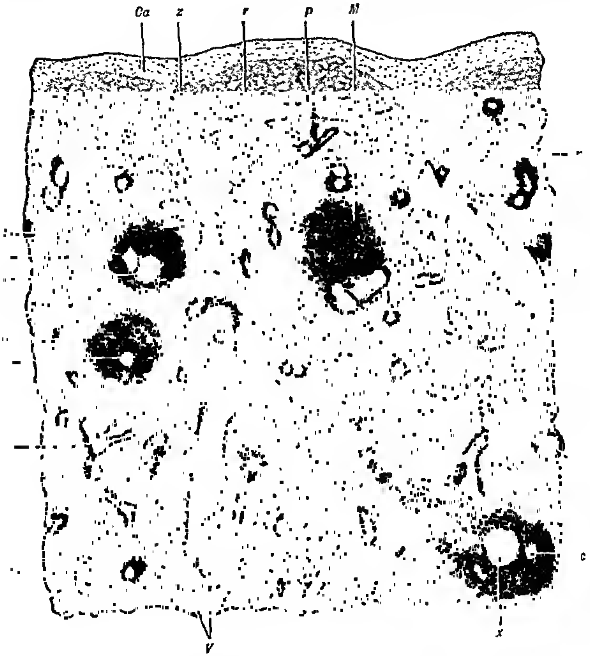

Fig. 239. Human spleen; section perpendicular to the serous surface. *A*, Artery in a trabecula; its branches at *a* are covered with a thin layer of lymphatic tissue (white pulp); those branches marked *ac* are surrounded by a thick layer of white pulp, *M*; *x*, lymphatic nodules; *Ca*, capsule; *p*, small penicilli; *r*, red pulp, penetrated by the pale venous sinuses (the blood in the sinuses and veins is not shown); *V*, veins in a trabecula communicating with the red pulp; *z*, origin of a trabecula, *t*, from the capsule. 32 ×. (A.A.M.)

The White Pulp. The white pulp (lymphatic tissue) forms a sheath about the arteries. The stroma is a network of reticular fibers closely joined to the primitive reticular cells and phagocytic reticu-

lymphocytes of various sizes, distributed to form diffuse and nodular lymphatic tissue. In the center of the lymphatic nodules of the spleen (Fig. 239, *x*) (see p. 80), as in the nodules of lymph nodes,

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the framework consists of very thin, scattered threads, while at the periphery it is coarser and much denser. A few elastic fibers are interspersed among the reticular fibers of the white pulp close to the artery and its capillaries.

The absolute and relative amounts of diffuse and nodular lymphatic tissue vary continuously and reflect the reaction of the lymphatic tissue to various generalized stimuli. The lymphatic tissue of the spleen undergoes the same changes described on pages 80-85 for the lymphatic tissue in general. That is, diffuse lymphatic tissue may become nodular and vice-versa. The volume and number of the nodules decrease progressively with age. In myeloid leukemia the red pulp is greatly increased in amount (besides changing qualitatively) while the white pulp almost disappears. In lymphatic leukemia, on the contrary, the white pulp hypertrophies and the red pulp atrophies. The amount of lymphatic tissue is said to diminish during starvation. Lymphocytopoietic centers in the nodules appear and disappear in connection with the general condition of the organism. In the young they are numerous, while in the aged they are usually absent (especially in man). "Reaction centers" are common in certain infections and intoxications.

The Red Pulp. This tissue fills the spaces between the terminal venous blood vessels, the so-called "venous sinuses," and appears in histologic sections as cords, the "splenic" or "Billroth cords" of tissue, running in all directions and forming a spongy framework. The red pulp is a modification of the lymphatic tissue of the white pulp. The red pulp gradually merges into the white; outside of the latter, there is a band of tissue looser than the white pulp and containing some erythrocytes but devoid of venous sinuses. It constitutes the so-called "marginal zone" of the periarterial lymphatic tissue sheath. Some authors include the venous sinuses in the red pulp.

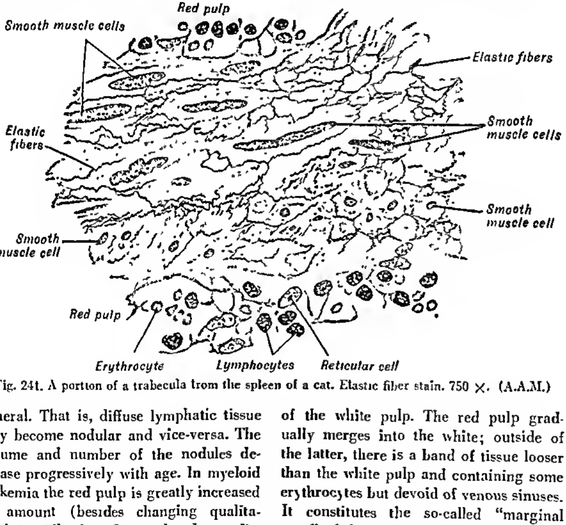

Fig. 241. A portion of a trabecula from the spleen of a cat. Elastic fiber stain. 750 ×. (A.A.M.)

A framework of reticular fibers forms the foundation of the red pulp. At the boundary between the white and red pulp, it is evident that the fibers of the former continue into those of the latter. The col-

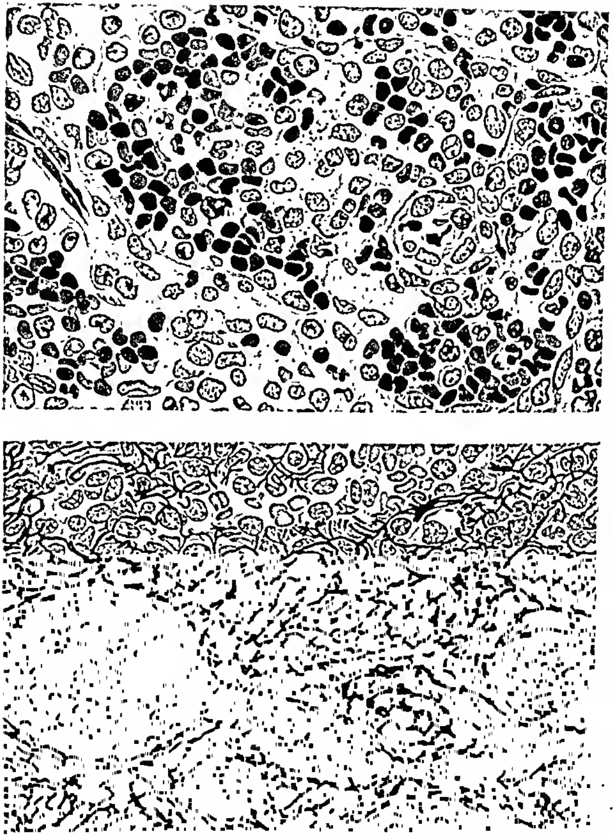

Fig. 240. Portions of two sections from a human spleen. The upper stained with hematoxylin and eosin, the lower stained with hematoxylin and eosin after the Bielschowsky impregnation method for reticular fibers. 600 ×.

different stages of digestion, and yellow and brown granules, some of which give an iron reaction (Fig. 244). Free macrophages can sometimes be found in the blood which fills the venous sinuses.

In many mammals (mouse, guinea pig, and hedgehog) and in human embryos the red pulp of the spleen contains small groups of myelocytes, erythroblasts, megakaryocytes, and plasma cells.

In infections, in some of the anemias and leukemias, in poisoning with certain blood-destroying agents, and in local inflammations of the organ, the splenic tissue undergoes a *myeloid metaplasia* (p. 112). Myelocytes, megakaryocytes, and erythroblasts develop within the red pulp; only myelocytes have been described as arising in the germinal centers. This indicates that both white and red pulp are composed primarily of the same lymphatic tissue, which also has myeloid potencies. The myeloid elements may develop from typical lymphocytes as well as from primitive reticular cells. The old idea of an "antagonism" between the red and white pulp is obviously untenable. The *red pulp is merely a modified lymphatic tissue which is heavily infiltrated with all of the cells of the circulating blood.*

Arteries. The branches of the splenic artery enter the hilum and pass along the

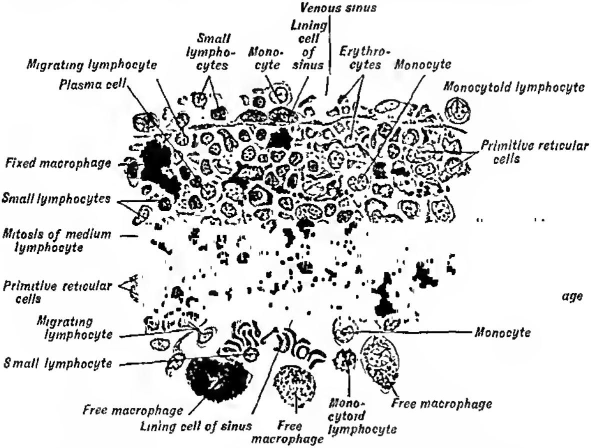

Fig. 243. Cross section of a cord of Billroth lying between two venous sinuses from the spleen of a rabbit injected with lithium carmine and India ink. Hematoxylin eosin-azure II. 460 ×. (A.A.M.)

trabeculae, with which they branch repeatedly, becoming progressively smaller in caliber. They are muscular arteries of medium caliber and have a loose tunica adventitia surrounded by the dense connective tissue of the trabeculae.

When the arterial branches have reached a diameter of approximately 0.2 mm. they leave the trabeculae (Fig. 245). At this place the tunica adventitia is replaced by a cylindrical sheath of lymphatic tissue which accompanies the ar-

lagenous fibers of the trabeculae continue directly into the reticular fibers of the red pulp. The fibrous stroma of the latter is accompanied by fixed macrophages and primitive reticular cells.

In the meshes of this framework are many lymphocytes, free macrophages and all the elements of the circulating blood. The nongranular leukocytes are the most numerous of these free cells. Among them small, medium-sized and large lymphocytes and monocytes are present in great numbers, intermingled without order. The various types of lymphocytes which arise in the white pulp spread by their ameboid movement throughout the red pulp where they continue to multiply.

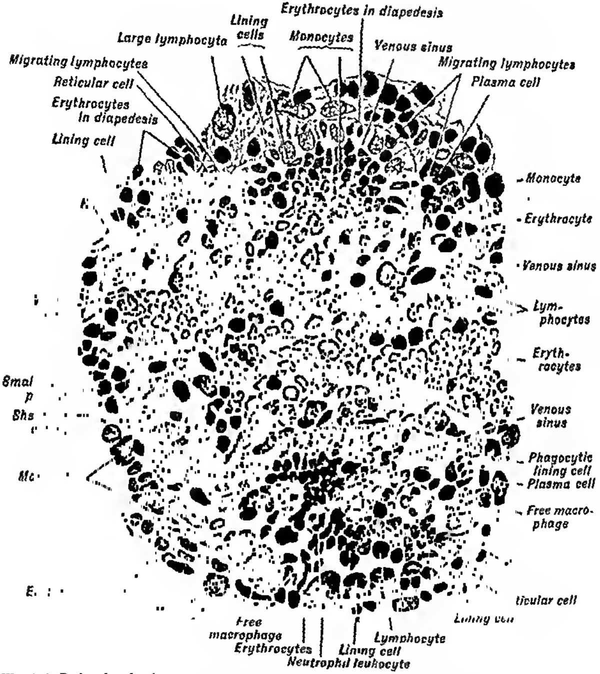

Fig. 242. Red pulp of a human spleen. *K*, condensed cytoplasm of lining cells. Eosin-azure stain. 750 ×. (A.A.M.)

The free macrophages are similar to those of the lymphatic tissue and are in close genetic relation with the fixed macrophages. They are round or irregularly shaped cells with large vesicular nuclei and much cytoplasm which often contains engulfed particles, mainly erythrocytes in

capillaries (Fig. 245), which either do not divide or split into only two branches. Their terminations are unknown and will be discussed after the veins have been described.

slightly developed. The tunica media is lost so that the sheath is external to the endothelium (Fig. 246). The sheath is a compact mass of concentrically arranged, elongated nuclei (probably reticular

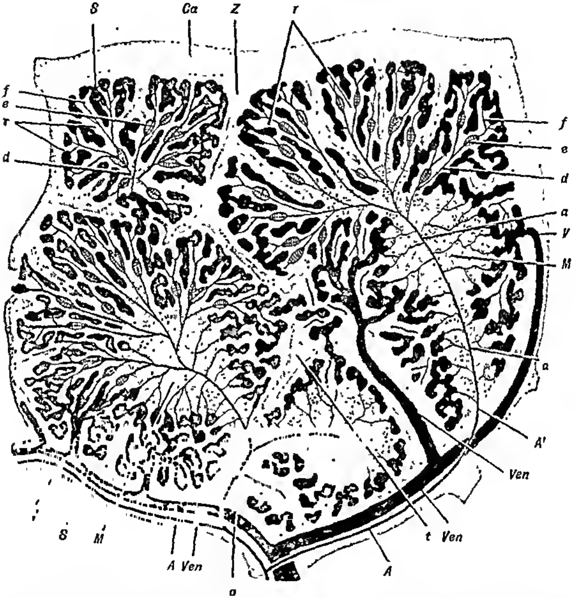

Fig. 245 Diagram of the spleen Two complete (right and left) and two incomplete (above and below) lobules are shown. A', Point where artery, A, leaves the trabecula; a, artery covered by a sheath of lymphoid tissue (white pulp); d, arteries of the pulp; e, sheathed arteries of Schweigger-Seidel; f, arterial capillaries; M, swelling of white pulp; r, red pulp; S, venous sinus; t, trabecula; V, veins entering trabeculae from red pulp; Ven, large veins in the trabeculae; Z, origin of trabecula from the capsule, Ca.

The artery of the pulp has a tunica media consisting of one layer of smooth muscles surrounded by a thin discontinuous envelope of lymphatic tissue which contains a few elastic fibers. In man the Schweigger-Seidel sheaths are only cells) and longitudinal fibers which continue into the reticular fibers of the red pulp. The arterial capillaries consist of the endothelium, supported externally by a few longitudinal fibers and elongated cells.

teries almost to the point where they break up into capillaries. The white pulp is the transformed tunica adventitia of the arteries and hence contains some elastic fibers. In many places along the course of the arteries the lymphatic sheath contains lymphatic nodules. The artery, although called "central artery" practically never passes through the nodules.

continue to branch and become thinner; on reaching a caliber of 40 to 50 μ they leave the lymphatic tissue and enter the red pulp. Here they branch into small, straight vessels called *penicilli*, which show three successive parts. The first portion is the longest (0.6–0.7 mm.) and is called the *artery of the pulp*, which rapidly becomes narrow and divides (the caliber

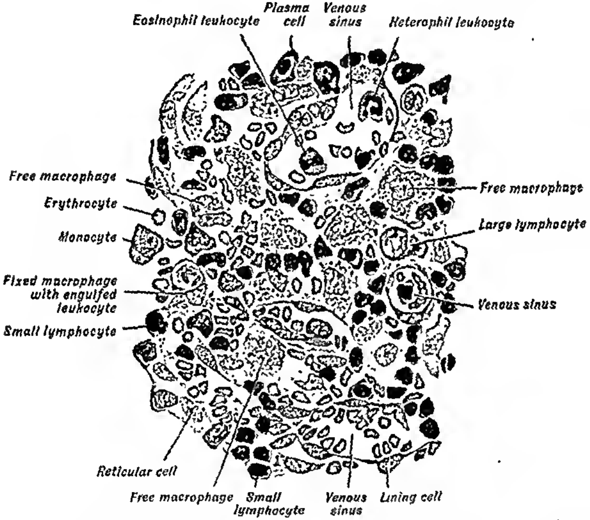

Fig. 244. Red pulp of a rabbit's spleen. The macrophages (free reticular cells) are loaded with hemosiderin granules. Eosin-azure stain. 750 ×. (A.A.M.)

Throughout its course within the white pulp, the artery gives off numerous capillaries which supply the lymphatic tissue of the sheath. The endothelial wall of these capillaries is supported externally by a thick network of reticular fibers. These arterial capillaries pass into the red pulp to unite with venous vessels (see Fig. 245).

The small arteries in the white pulp

now is about 10 μ). Each branch (0.15–0.25 mm. long) is provided with a characteristic spindle-shaped thickening of its wall, the *Schweigger-Seidel sheath*, but has a very narrow lumen (6–8 μ)—the so-called *sheathed artery;* this portion may ramify into two or three branches. These—forming the third portion—are the shortest (60 to 90 μ with a lumen up to 10 μ) and represent simple arterial

the venous sinuses of the spleen is perforated by many permanent openings. The solution of this problem will do much to solve the riddle of the blood circulation in the spleen (see below).

In the sheep and ox the wall of the sinuses is composed of an exceedingly irregular network

densed stroma of the red pulp and a few elastic fibers. These pulp veins coalesce to form the veins of the trabeculae. These vessels consist only of endothelium supported by the connective tissue of the trabeculae. The trabecular veins form the splenic veins which leave the organ at the hilum and empty into the portal vein.

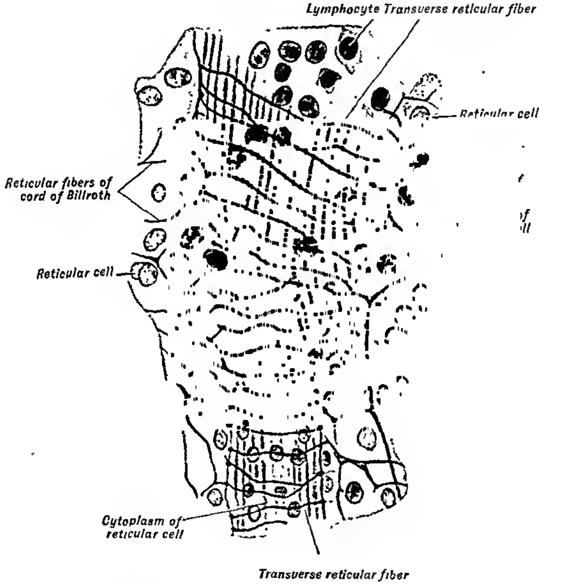

Fig. 217. Wall of a venous sinus from a monkey's spleen, seen from the surface. Redrawn after Mollier.

of reticular cells and fibers indistinguishable from the stroma of the red pulp There are other variations in different species. The venous sinuses thus appear to be more or less regular cylindrical meshes of the reticular framework of the tissue.

The venous sinuses empty into the veins of the pulp whose wall consists of endothelium supported externally by a con-

The Union of the Arteries with the Veins. In almost all the other organs of the body the connection between the arterial and venous systems is accomplished by a direct passage of the arterial capillaries into the venous, in which the endothelium retains its continuity and the vascular lumen is completely closed. In the

In the dog, hedgehog and pig, and in the lower vertebrates, the sheaths are thick, oval bodies; they may be seen in the red pulp with low magnification. Red corpuscles are always present in large or small numbers inside the sheath.

Veins. The veins of the spleen begin as networks of *venous sinuses* which penetrate all of the red pulp and are especially numerous about the white pulp. These vessels are called *sinuses* because they have a wide (12 to 40 μ) irregular lumen each of these rod-shaped cells is distended by a nucleus. These lining cells are fixed macrophages identical in origin and properties with those of the adjacent splenic cords, although normally less actively phagocytic than the fixed macrophages of the cords.

Outside the rod-shaped cells, the wall of the sinus is supported by a system of circular, occasionally branching, reticular fibers which continue into the reticular

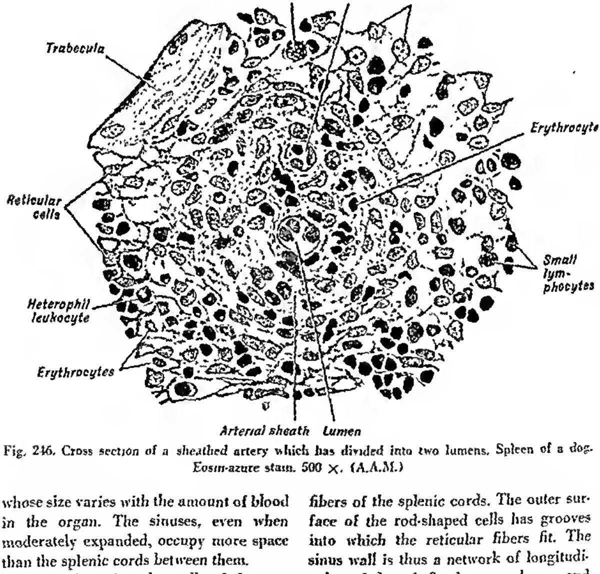

Fig. 246. Cross section of a sheathed artery which has divided into two lumens. Spleen of a dog. Eosin-azure stain. 500 ×. (A.A.M.)

whose size varies with the amount of blood in the organ. The sinuses, even when moderately expanded, occupy more space than the splenic cords between them.

Unlike the veins, the walls of the venous sinuses do not contain common vascular endothelium but are lined by long narrow cells arranged parallel to the long axis of the vessel. Adjacent cells are separated by slitlike spaces and their bodies project far into the lumen. The middle of fibers of the splenic cords. The outer surface of the rod-shaped cells has grooves into which the reticular fibers fit. The sinus wall is thus a network of longitudinal, rod-shaped fixed macrophages and circular reticular fibers. Some hold that the meshes of this framework are closed by a thin, homogeneous membrane or by the edges of the phagocytes. Others claim the existence of such a membrane has not been clearly proved and that the wall of

found passing through the walls of the venous sinuses. It is difficult to reconcile this finding with either the "closed" or the "open" circulation theories, for these views hold that there are open connections between the sinuses and the arterial terminals or the meshes of the cords of Billroth respectively. It is of course possible that such pictures are artefacts due to the collapse of the spleen after it is incised before being fixed.

The problem of circulation in the spleen would seem to be an ideal one for solution by direct observation of the living organ. Unfortunately, the technic available for this is difficult and the two extensive reports made with it are contradictory. According to one the circulation in the spleen is closed, there is a marked intermittence of circulation, there is extensive filtering of the liquid portion of the blood from the sinuses into the cords of Billroth, and diapedesis of erythrocytes from the sinuses occurs frequently during the death of the animal. These conclusions are contradicted by the latest study on living spleens, which finds that the circulation is open, that is, without preformed connections between the arterial and venous systems, so that the blood from the terminals of the arterial tree passes between the reticular cells (fixed macrophages) of the cords of Billroth and finds its way through openings into the sinuses. According to these observations, erythrocytes may be stored in the spaces between the reticular cells and it is here that the separation of the blood cells from the plasma occurs. The channels in the cords of Billroth vary from time to time with the degree of engorgement of that part of the organ, so that a channel which previously had been a tortuous passage between reticular cells may appear as a direct communication to the lumen of a venous sinus when the spleen is contracted. From the above it is obvious that the manner of connection of arterioles and venules in the spleen requires further investigation. For the time being, it appears that the weight of evidence is in favor of an open circulation.

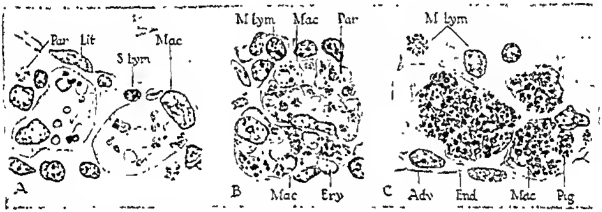

Fig. 219. Sections of spleens of monkeys at different stages in malarial infection (P. brasilianum). Sluggish phagocytosis by macrophages during acute rise in the infection (A), intense phagocytosis of parasitized erythrocytes during the crisis (B), and accumulation of malarial pigment and débris of erythrocytes in the macrophages two days after the crisis (C); *Adv*, adventitial cell; *End*, endothelium of a venule; *Ery*, erythrocyte; *Lit*, lining cell of sinus; *Mac*, macrophage; *M Lym*, medium lymphocyte; *Par*, parasite; *Pig*, pigment; *S Lym*, small lymphocyte. Hematoxylin-eosin-azure II. After Taliaferro and Cannon. 970 ×.

If the splenic veins are tied for a few moments, the splenic artery ligated and the entire organ fixed and sectioned, one can easily trace columns of erythrocytes from the meshes of the cords of Billroth into the venous sinuses. The pictures seen

spleen, however, the connection is different and its details are still subject to dispute. There are three main theories as to how blood gets from the arteries to the venous sinuses: (1) The arterial capillaries open directly into the spaces between the reticular cells of the splenic cords and the blood gradually filters into the venous sinuses—the "open" circulation theory. (2) The arterial capillaries communicate directly with the lumen of the venous sinuses—the "closed" circulation theory. (3) The compromise view holds that both types of circulation exist at the same time. One of the latest aspects of this theory is that a "closed" circulation in a contracted spleen may become an "open" circulation when the organ is distended. (See Fig. 248.)

The opposing theories are based on the following observations: (1) There are always many erythrocytes scattered irregularly between the fixed cells in the splenic cords. As there is no evidence of erythropoiesis in the cords, the conclusion is that the red blood cells have come from the circulating blood through gaps in the vascular connection between the arterioles and the venous sinuses. Those who maintain that the circulation is "closed" hold that the number of erythrocytes in the splenic cords is much smaller than it should be if the arterial capillaries opened directly into the pulp. They point out that if the capillaries were open, the red pulp should be completely filled with blood, as in hemorrhages in the spleen.

(2) When the splenic arteries are injected, even at low pressures with stained fluids, India ink, or avian erythrocytes, the foreign materials readily gain access to the spaces between the fixed cells of the splenic cords, particularly in the red pulp about the white pulp. Only later do they reach the venous sinuses. When the splenic vein is injected, the venous sinuses and the meshes of the stroma can be filled easily, but the arteries cannot.

Those who hold for a closed circulation believe this injection of the red pulp by foreign materials is artificial and results from the rupture of the delicate vascular walls.

(3) In every freshly fixed spleen, granulocytes, lymphocytes with greatly constricted nuclei and erythrocytes can be

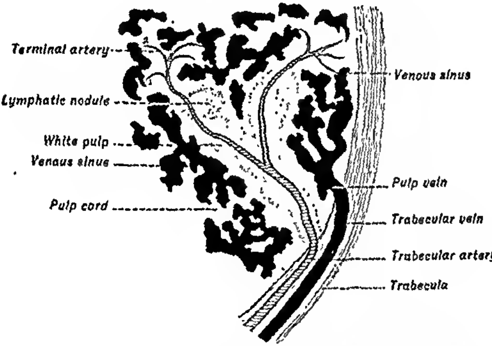

Fig. 248. Diagram to show closed (1) and open (2) circulation through the spleen.

hemoglobin or certain iron salts is introduced under the skin or into the abdominal cavity of normal animals (pigeon, guinea pig), the amount of iron-containing pigment in the reticular cells of the spleen is greatly increased. If the same is done to splenectomized animals, the iron is rapidly excreted through the liver and intestine.

Probably correlated with these phagocytic functions, is the great importance of the spleen in the production of antibodies and in the defense of the organism against various infections, especially those which are generalized enough to be in the blood stream. The liver and bone marrow, which contain macrophages similarly strategically placed for contact with substances in the blood, share these functions. Of these three organs, the macrophages of the spleen are generally most active, those of the liver next and those of the bone marrow least. The direct function of the macrophages in defense is well exemplified during a blood infection such as malaria. Here from the onset of the infection parasites are filtered out in the spleen, liver and to a less extent, bone marrow, and are eventually phagocytosed by the macrophages of these organs. Then as acquired immunity develops, they are filtered out and phagocytosed so much more rapidly that they not only become exceedingly scarce but superinfection is prevented. This acquired immunity is dependent on an increased number of macrophages, particularly in the spleen, and a very much greater individual activity of the macrophages in all three organs. It seems probable that the increase in individual phagocytic activity in acquired immunity is associated with an opsonic antibody. Whenever the malarial infection persists a pronounced hyperplasia of several types of cells of the spleen occurs. Mitotic division is most pronounced in the medium lymphocytes, to a less extent in the large lymphocytes and reticular cells, and to a very slight degree in the functional macrophages. This type of proliferation has been termed "mesenchymal activation" and is frequently associated with pronounced splenomegaly. A similar activation has been noted in infections other than malaria where the exact causal agent is unknown and where direct phagocytic activity can accordingly not be observed, and during immunization with noninfec-

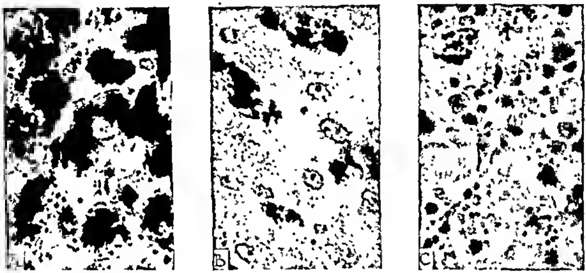

Fig. 250. Sections of spleen (A), liver (B), and bone marrow (C), of monkey infected with malaria (P knowlesi). Phagocytosis of the black malarial pigment is most marked in the spleen The Kupffer cells in the liver are also heavily laden with pigment. The phagocytosis is less prominent in the bone marrow. Photomicrographs. After Taliaferro and Mulligan. 830 ×.

in spleens prepared by this old method correspond more with those seen in the living organ than do the usual preparations made by cutting thin slices from the fresh organ before fixation.

Lymphatic Vessels and Nerves. In man lymphatic vessels are poorly developed and are found only in the capsule of the spleen and in the thickest trabeculae, particularly those in the vicinity of the hilus. In some mammals true lymphatic vessels follow the arteries of the white pulp to the hilus. Nervous networks, which originate from the celiac plexus and which consist almost entirely of nonmedullated fibers, accompany the splenic artery and penetrate into the hilus of the spleen. In the sheep and ox these nerves form trunks of considerable thickness. The nerve bundles follow mainly the ramifications of the arteries and form networks which can be followed up to the central arteries of the white pulp and even along the branches of the penicilli. The terminal branches usually end with button like thickenings in the smooth muscles of the arteries and of the trabeculae. Apparently many branches penetrate into the red as well as the white pulp, but their endings here are not definitely established.

The Functions of the Spleen.

The spleen is closely related to the lymphatic and hemal nodes and the bone marrow, and is an important hemopoietic organ. Lymphocytes are produced in it, mainly in the white pulp and in particular in its nodules. From the white pulp they migrate into the red pulp, where some of them are thought by certain authors to become monocytes. Lymphocytes and monocytes actively enter the venous sinuses through the reticular wall.

In the embryo the spleen is a hemopoietic organ of some importance (p. 104). The red corpuscles of the splenic tissue of the *normal adult man* are never formed in the white or the red pulp. In certain mammals (but not in man), a few myelocytes are found normally in the red pulp.

In pathologic cases, especially in *myeloid leukemia*, the red pulp of the spleen undergoes myeloid metaplasia (p. 112). In this case a large number of erythroblasts, megakaryocytes, and myelocytes of various kinds appear in the tissue, so that the red pulp acquires a structure very similar to that of red bone marrow.

After the removal of the spleen the number of lymphocytes in the blood increases (lymphocytosis); this is explained by an excessive compensation on the part of lymph nodes. Then, there is an increase in the number of eosinophil leukocytes. Both phenomena soon disappear.

The spleen also acts as a store for red blood cells. From time to time large numbers of them are retained in the red pulp and then given up to the blood stream as they are needed in the circulation.

The rate of circulation of the blood through the red pulp may be fast or slow, and even stops for varying periods of time. This allows the macrophages many opportunities for removing substances and particles (including bacteria) from the passing blood.

The destruction of erythrocytes occurs in the spleen, with a varying intensity in different species, for they are phagocytosed by the macrophages in the splenic cords and sometimes by those lining the sinuses. Disintegrating erythrocytes and granules of hemosiderin are often found in the cytoplasm of these phagocytes. After poisoning with substances which destroy the red blood cells (pyrogallol), the red pulp becomes filled with large macrophages containing the débris of erythrocytes. The destruction of erythrocytes also proceeds extracellularly, for particles of disintegrating erythrocytes may be encountered among the cells of the red pulp (p. 98). After splenectomy, the erythrolytic function is carried out by the macrophages of the bone marrow, lymph nodes, and liver.

Closely connected with erythrocyte destruction by the spleen is its function in iron metabolism. The iron-containing component of hemoglobin is freed from the disintegrating erythrocytes and is stored in the reticular cells of the spleen. This accumulated iron is again utilized in the formation of hemoglobin. The site of this latter process is unknown. If a solution of

tive. Similar results have been obtained with certain normal serums which are trypanocidal and even with various protective immune serums. In the last case splenectomy probably removes macrophages which normally phagocytose opsonized parasites.

content of macrophages and lymphoid cells.

When the lipoids in the blood are increased in amount, the reticular cells of the spleen, like the other macrophages of the body, have the capacity to remove the lipoids from the blood and to store them. During this process these macrophages increase greatly in size, are filled with

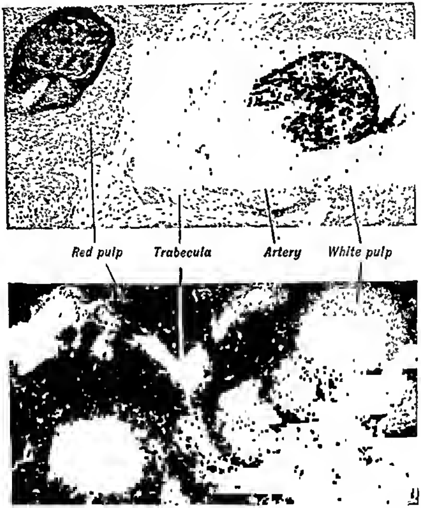

Fig. 252. Photomicrographs of sections of spleen of a dog, showing distribution of alkaline phosphatase as the black stained material in the white pulp of the upper figure and of acid phosphatase as the black stained material in the red pulp of the lower figure. The very thick trabeculae are characteristic of the dog's spleen. Courtesy of G. Gomori. 30 ×.

As macrophages in contact with the blood stream are not restricted to the spleen, it is not surprising that the effects of splenectomy largely disappear as the splenic functions are asumed by the macrophages of other organs, particularly the liver and bone marrow. This makes very improbable the view of a few authors that the spleen possesses peculiar powers of defense in addition to those referable to its

lipoid droplets and acquire a foamy appearance; this is observed in man in diabetic lipemia and in lipoid histiocytosis (Niemann-Pick disease), and in the experimental hypercholesterolemia of rabbits.

The spleen is thought by some to regulate the formation and destruction of erythrocytes by the production of a hormone which decreases the erythropoietic capacity of the bone marrow. Others think that a hormone is produced by the spleen which inhibits the formation of leukocytes in the other hematopoietic organs.

tious antigens. In malaria the functional significance of the activation is largely that of producing increased numbers of macrophages. Taliaferro and Mulligan (1937) have shown that in malaria numerous lymphocytes can be found in all stages of development into functional macrophages and that hyperplasia of the reticular cells (fixed macrophages) is insufficient to describe the cellular mechanism involved in immunity.

As would be expected from these histologic findings, removal of the spleen sometimes lowers the natural resistance or the immune response. The conflicting evidence on this problem is probably due to the variations in different species of the spleen-weight to body-weight ratio and also to the compensatory action of other organs after one or two weeks; but in general, rats, mice, and dogs having large spleens show greater effects than guinea pigs and rabbits with their comparatively small spleens. In many animals splenectomy is often followed by a recrudescence of a latent or low grade infection, as is strikingly exemplified by Bartonella infections of rats, piroplasms of dogs and sheep and malaria of monkeys. Similarly, splenectomy often temporarily depresses antibody formation. This effect is greatly enhanced if combined with so-called "blockade" by the intravenous injection of colloidal dyes or particulate matter.

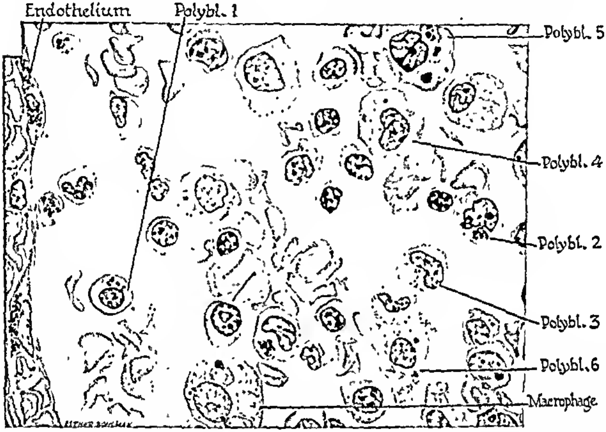

Fig. 251. Transitional forms (*Polybl. 1–5*), showing progressive hypertrophy and phagocytosis, *from nongranular leukocytes to macrophages. A small trabecular vein from spleen of monkey (Silenus* rhesus) killed nine days after infection with malaria (P. knowlesi) and three days after the parasites appeared in the peripheral blood. *Polybl. 1* has a small lymphocyte nucleus with an increase in cytoplasm *Polybl. 5* has a medium-sized lymphocyte nucleus. The endothelium of the trabecular vein (actually a pulp vein) is phagocytic. Hematoxylin-eosin-azure II. After Taliaferro and Mulligan. 1400 ×.

An intact macrophage system, a large part of which is in the spleen, seems necessary for the full utilization of specific drugs used in the treatment of infections due to trypanosomes, spirochetes, and bacteria. Thus, if the spleen is removed, and particularly if blockade accompanies *splenectomy*, a dose of drug effective in curing a normal mouse is no longer effec-

lular Reactions during Primary Infections and Superinfections of *Plasmodium brasilianum* in Panamanian Monkeys, Jour. Inf. Dis., (59), 72, 1936.

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During digestion, the spleen increases in size. The reason for this is not known. Relations between the spleen and the various glands of internal secretion have not been established.

Histogenesis and Regeneration of the Spleen. The primordium of the spleen appears, in human embryos of 8 to 9 mm., as a small thickening of the dorsal mesentery, consisting of a closely aggregated mass of energetically multiplying, mesenchymal elements.

The mesenchymal cells which compose this first primordium of the spleen multiply independently by mitosis and the primordium grows. It has been supposed that it also increases in size by apposition of new cells from the mesothelium of the body cavity covering the primordium. After the embryo (pig) has reached a length of 15 mm., it receives no more cells from the mesothelium.

The elements of the primary mesenchymal primordium differentiate in two directions. Some remain connected with one another by means of processes and form the reticular framework of the white as well as of the red pulp. Some of the mesenchymal elements soon become isolated from the rest and become free cells, located in the meshes of the framework. At first they all have the character of basophil wandering elements—lymphocytes. Later on, they give origin to red corpuscles, granular myelocytes and leukocytes, and megakaryocytes, as well as to more lymphocytes. In the lower vertebrates up to the urodele amphibians, this erythropoietic function is retained throughout life in the spleen; in the higher vertebrates the myeloid function stops sooner or later and is replaced by an erythrolytic function, although the formation of lymphocytes persists throughout life.

In mammals (pig) the mesenchymal primordium contains a capillary vascular network connected with the afferent arteries and efferent veins Meanwhile, irregular spaces, the precursors of the venous sinuses, appear (embryo pigs of 4 to 6 cm.) and become connected, in 6 to 7 cm. embryos, with the afferent and efferent vessels.

The tissue of the embryonic mammalian spleen has at first a myeloid character and cannot be compared with either the red or white pulp. At the end of fetal life (in the rat) the adventitia of the arteries begins to be infiltrated with large numbers of lymphocytes and in this manner the white pulp originates; typical lymphatic nodules are found after birth. Simultaneously, the myeloid elements which had reached their maximum development three weeks after birth (in the rat) begin to disappear gradually, and the tissue of the spleen located between the accumulations of white pulp may be then called the red pulp

When the spleen is removed, its functions are taken over by other organs, and the formation of a new spleen has never been observed, although a compensatory hypertrophy of the so-called "accessory spleens" has been described. Local injuries and wounds of the spleen are accompanied by a temporary myeloid metaplasia of the red pulp and heal with a simple scar. In the amphibians, particularly in larval stages, a certain degree of regeneration is possible, while in birds the spleen shows marked regenerative powers.

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of the cellular products have not been found.

The ovary and testis are examples of *cytogenous glands* in which parts of the secretion are the living germ cells. In the same group might be placed, perhaps, the hematopoietic tissues.

The type of secretion in which the glandular cell remains intact throughout a cyclic process of formation and discharge, and then formation again, followed by discharge, and so on, of secretory products, is called *merocrine secretion.* In *holocrine secretion* the products accumulate within the cell body; the cell finally dies and is discharged as the secretion of the gland, new cells having arisen in the meantime to repeat the same cycle. The intermediate type of secretion is the so-called *apocrine type.* Here the secretion accumulates within the free end of the cell; after a time, this portion of the cytoplasm is pinched off but the nucleus and most of the cytoplasm are undamaged, and after a recovery period, the cell passes through the same process again.

The details of these various types of secretion will be considered with the descriptions of the specific organs: merocrine secretion with the salivary and pancreatic glands, holocrine secretion with the sebaceous glands, and apocrine secretion with the mammary gland.

Unicellular Glands. In the mammals practically the only type of unicellular gland is the *mucous* or *goblet cell* which secretes mucin, a protein which forms with water a lubricating solution called *mucus.* These cells are scattered on many mucous membranes, especially those covered with columnar or ciliated epithelium (Fig. 29). A fully developed mucous cell has an oval apical, and a slender basal end; it resembles a goblet. The dilated part consists of a thin protoplasmic wall usually called the *theca* and a cavity which is filled with an almost homogeneous clear mass with a multitude of pale droplets of *mucigen.* This material is very difficult to preserve in fixed preparations (see Hempelmann for details on staining). At the free surface of the cell the theca is interrupted and through this opening the mass of mucigen often protrudes in the form of a plug. The stalk of the goblet cell contains a more or less compressed and disfigured nucleus.

The droplets of mucigen leave the goblet cell through the opening on the surface, dissolve at once and are transformed into mucin. This elimination of the mucigen may proceed gradually and the cell may keep its goblet form for a long time. In other cases the whole content is thrown out at once and the emptied cell collapses and is compressed between the neighboring epithelial cells (Fig. 29, *b*). After a while a new accumulation of mucigen may begin in the same cell. Small granules appear above the nucleus in the region of the Golgi net; at first they stain with acid dyes and do not swell easily on the addition of water. They gradually enlarge, acquire the character of mucigen, and cause a new swelling of the apical part, while the cytoplasm between them is reduced to thin partitions and finally may disappear.

A goblet cell seems to pass many times through the successive phases of secretory activity until it finally perishes and is shed. Mitoses have been observed occasionally in them. As a rule, however, new goblet cells arise through a transformation of indifferent epithelial cells or cells with a striated border or cilia. The transformation of a goblet cell into a common epithelial cell seems doubtful.

Multicellular Glands. If the glandular cells in an epithelial sheet replace all the common epithelial cells, *a secretory epithelial surface* results (Fig. 253, *b*). In the mammalian body, the epithelium of the chorioid plexuses and the surface epithelium of the gastric mucosa with its foveolae are of this type; the epithelium of the mucous membrane of the uterus and oviducts, to a certain extent and at certain times, also belongs to this category.

A transitional step between the unicellular and multicellular glands is represented by the *intra-epithelial glands* (Fig. 251). They are found

Chapter XIII

GLANDS

We have seen that the cells forming the connective substance and the nervous, muscular and vascular systems have completely lost the arrangement of the primitive epithelial sheets from which they were derived. In the organs described in the following chapters the epithelium persists as such or as special structures called *glands*. It was pointed out in the chapter on epithelium that the most important and general function of the epithelium is its participation in the metabolism of the body through the absorption of substances from the outside medium, their modification in the body, and the elimination of other materials to the outside. Practically all substances which are normally received and given off by the body must pass through an epithelium. The main function of the glands is the transformation by processes of *secretion* of the materials brought to them into products which are to be used by other cells of the body or eliminated. In this process, work is done (see discussion by Flexner, 1934). Some authors distinguish *secretion* from *excretion* and use the latter to mean the simple separation of unchanged waste materials—a process which does not require work on the part of cells. However, it is often difficult to decide whether a particular process is one of secretion or excretion or both. Examples of the problem will appear in many of the organs described in the following chapters.

The secreting or glandular cells may be scattered singly as *unicellular glands* among the other cells in the epithelial sheet. If all of the cells in the sheet are glandular they form a *secretory epithelial sheet*. The mechanism for secretion reaches its highest development in the *multicellular glands*, in which large numbers of secreting cells are concentrated in a small space and form a well delimited organ with a special structure. All multicellular glands arise as invaginations of the epithelial sheet into the underlying vascular connective tissue, from which they are usually separated by a basement membrane.

Most of the glands elaborate an *external secretion* (open or *exocrine glands*). In these the glandular cavities open freely on the surface of the epithelium from which they have developed and the secretion is poured out on this surface. These exocrine glands may be simple or complex. Other glands have an *internal secretion* (*closed* or *endocrine glands*). In the embryo the latter originate in the same way as the open glands through the invagination of an epithelial sheet. Later, however, the connection with the sheet is severed and the secretion passes into the blood or lymph vessels of the gland and is distributed in this way all over the body (p. 293). In the majority of endocrine glands, the original epithelial arrangement of the cells is completely lost.

In mucous cells (p. 289), many enzyme-forming cells (salivary glands, pancreas, fundic glands), and some of the endocrine glands there are morphological evidences of secretion. In many other glands, however, visible indications of the formation

tions; they are the most important functional part of the organ. The parts of the gland which serve merely for the elimination of the secretion are called *excretory ducts.* Here the epithelium has a simple structure and often remains undifferentiated. In some cases, however, as in *the salivary glands, certain stretches* of the duct system are believed to be secretory.

The free surface of the glandular cells is usually provided with terminal bars In many glands the secreting surface is increased by many extremely fine canals, the *secretory capillaries,* which arise from the lumen of the terminal portion and penetrate between the sides of the glandular cells. They are often branched and end blindly before reaching the basement membrane. They have no wall of their own, but are formed by groovelike excavations in adjoining cells (Fig. 255).

The secretory capillaries can be well demonstrated with the aid of the Golgi impregnation method, by which the lumen of the terminal portions and the secretory capillaries are stained black. Iron hematoxylin, which stains the terminal bars black, shows that these cement lines penetrate the secretory capillaries and give them a very characteristic aspect in longitudinal and in cross section (Fig. 34).

Exceptionally, glandular cells may contain an *intracellular* system of fine canaliculi which seem to drain the secretion (parietal cells of the gastric glands, Fig. 340).

In the great majority of glands the epithelium lining the glandular cavities is separated by a basement membrane from the connective tissue with its blood vessels. In certain cases, however, the glandular epithelium is intimately penetrated by networks of blood vessels accompanied by connective tissue.

A discussion of the formation and elimination of secretion granules is found in Chapter XVI.

Between the glandular cells and the basement membrane in the sweat and salivary glands are

Fig. 254. Intra-epithelial gland from the pseudostratified ciliated epithelium of the laryngeal surface of the epiglottis, of a woman of seventy-two years. 534 ×. After V. Patzelt, from Schaffer

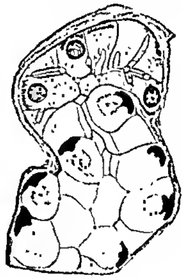

Fig. 255. Section of terminal portion of the mandibular gland of man; it contains clear mucous and darker albuminous cells. Between the latter, secretory capillaries and systems of terminal bars, stained black with iron hematoxylin. Redrawn after Zimmermann.

spindle-shaped or branched cells with a fibrillar cytoplasm They are called *basket* or *myoepithelial* cells; their supposed contractions are believed to facilitate the emptying of the terminal portions.

in the human body in the pseudostratified or stratified columnar epithelium of the nasal mucosa and the adjoining areas, of the caruncula lacrimalis, of the ductuli efferentes and of the urethra. They are small accumulations of glandular (usually mucous) cells within the epithelial sheet; they have a small lumen; they do not cause the basement membrane to project into the connective tissue.

The great bulk of those organs usually called "glands" is composed of secretory underlying connective tissue (Fig. 253, *c*). *The epithelium which lines the inner* surface of the invaginated area assumes a secretory character. In the simplest case the invagination is a hollow sac whose cavity is called the *glandular cavity*. The glandular epithelium everywhere remains sharply separated from the surrounding connective tissue by the invaginated basement membrane.

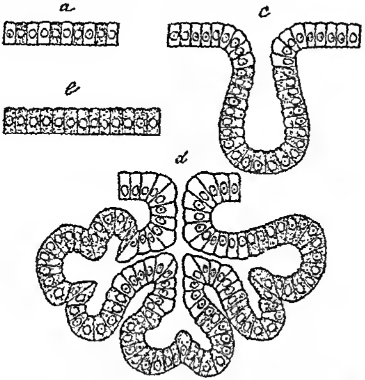

Fig. 253. *Diagram of uni- and multicellular glands: a,* Granular, glandular cells are scattered singly among clear, common epithelial cells; *b,* glandular cells arranged in a continuous sheet—secretory epithelial surface; *c,* simplest type of a multicellular gland: the area lined with glandular cells forms a saclike invagination into the subjacent tissue; *d,* multicellular gland of greater complexity: the glandular spaces are lined partly with glandular cells (terminal portions), partly with common epithelium (excretory ducts).

cells and only to a minor extent of the supporting connective tissue with its contained vessels and nerves.

Two factors can be distinguished in the development of the multicellular glands—an increase in the number of secreting cells and their progressive structural and functional differentiation. In the vertebrates an increase in the number of cells usually precedes the differentiation of special structures.

All multicellular glands arise as invaginations of the epithelial sheet into the

From this primitive form all the types of multicellular glands are derived. New centers of growth in the wall of the sac bring about secondary, then tertiary and further invaginations. Thus an increasingly complicated system of branching glandular cavities arises.

The epithelial lining of the glandular cavities differentiates in two directions. The cells which elaborate the secretion are usually concentrated in a single layer in the blind ends of the glandular cavities. These are the *secretory* or *terminal por-*

portion is a long coiled tubule which passes into a long excretory duct (Fig. 256, *b*). The sweat glands belong to this category. In the large axillary sweat glands (of apocrine type), the coiled terminal portions branch.

3. *Simple Branched Tubular Glands.* The tubule of the terminal portion is split forklike into two or more branches which are usually slightly coiled near their ends (Fig. 256, *d*). An excretory duct may be absent, as in the glands of the mucous membrane of the stomach or of the uterus, or there is a simple short excretory duct, as in some of the small glands of the oral cavity, the tongue, and the esophagus, and in some of the glands of Brunner (Fig. 256, *c*).

4. If the terminal portion has the form of a spherical or elongated sac, the gland is called *acinous* or *alveolar* (Fig. 256, *e*). If only one acinus is present with one excretory duct, it is a *simple acinous gland*; this type does not occur in mammals. If the acinus is subdivided by partitions into several smaller bodies (Fig. 256, *f*), or if several acini are arranged along a duct (Fig. 256, *g*) it is a *simple branched acinous gland* (sebaceous glands of the skin, glands of Meibom in the eyelids).

Compound exocrine glands. A compound gland consists of several or many units, called *lobules*, each of which corresponds to a simple gland. Thus, a small lobule of the mandibular gland corresponds to a small gland of the mucous membrane of the cheek. A compound gland consists, then, of a varying number of simple glands whose small excretory ducts join to form ducts of a higher order, which in turn combine with other ducts of the same caliber to form larger ducts of a still higher order, and so on. The lobules of succeeding orders can be seen without a microscope.

The compound exocrine glands are sometimes classified by the secretion they furnish. Thus mucous, albuminous, and mixed glands are distinguished. This, however, can be applied successfully only to the glands of the oral cavity. Sometimes the outer form of the terminal portions may serve as the distinguishing criterion. The drawbacks here are that it is very difficult to determine the form, even by reconstructing models or by teasing, and that the different forms are all connected by transitions.

1. In *compound tubular* glands the terminal portions in the smallest lobules are more or less coiled, blindly ending, usually branching, cylindrical tubules (Fig. 257). To this category belong the pure mucous glands of the oral cavity, glands of the cardia, some of the glands of Brunner, the bulbo-urethral and vestibular glands, the kidney. In some special cases, as in the testis, the coiled terminal portions anastomose with one another and form loops.

2. In the *compound acinous* or *alveolar* glands the terminal portions are supposed to have the form of oval or spherical sacs. However, as a rule, the form is that of irregularly branched tubules with numerous saccular outgrowths on the wall and on the blind ends (Fig. 257). These glands, therefore, should be designated as compound tubulo-acinous. To this group belongs the vast majority of the larger exocrine glands—the albuminous and mixed glands of the oral cavity and respiratory passages, and the pancreas.

In some cases the excretory ducts which lead from the lobes of a gland do not all join into a single main duct, but open independently on a restricted area of a free epithelial surface (lacrimal, mammary, and prostatic glands).

In all simple and compound glands the terminal portions and the excretory ducts are arranged in all directions. Hence, histological sections never show such simple diagrammatic relations as those in Fig. 257. They only show, as a rule, glandular cavities of irregularly rounded form which are lined by epithelial cells resting on a basement membrane and are separated from one another by partitions of connective tissue containing blood vessels and nerves (Fig. 255). The shape of the cavities, their connections with one another and with the free epithelial surface can only be elucidated through teasing the macerated tissue or with the aid of plastic reconstruction.

Endocrine Glands. In the section on the nervous tissue it was pointed out that widely separated organs and tissues are brought into coordinated movements and activities through the mediation of the central and autonomic nervous systems. However, influences arising in one organ may affect other organs or even the whole body in another way. This mechanism consists in the elaboration of particular

The interstitial tissue of the glands is loose connective tissue. In different glands its content of cells may vary greatly. The blood capillaries usually form dense networks on the outer surface of the basement membrane. Lymph capillaries are usually found in great numbers in the interstitial tissue. Besides the vasomotor nerves, all glands probably possess specific secretory nerves which ordinarily form netlike plexuses on the outer surface of the basement membrane. Branches of the plexus penetrate the membrane and form, on its inner surface, another plexus. From the latter, very thin fibers arise which end freely between and on the surface of the glandular cells.

oval body with a narrow lumen it is an *acinus.* The terms "alveolus" and "acinus," however, are used as synonyms by many authors. The form of the terminal portion, however, if taken as the sole criterion, does not give a satisfactory classification. The general architecture and structure of the organ must also be taken into consideration. As mentioned above, glands are also classified as exocrine or endocrine.

Exocrine Glands. In the exocrine glands the epithelium lining the glandular cavities is demarcated by a basement membrane from the connective tissue and the blood vessels. Simple and compound exocrine glands can be distinguished.

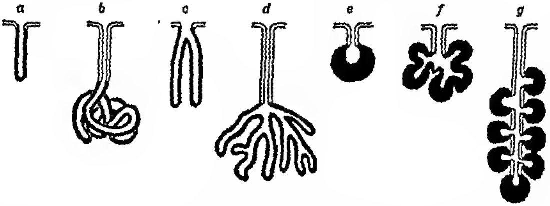

Fig. 256. Diagrams of simple exocrine glands. *a*, Simple tubular; *b*, simple coiled tubular; *c*, *d*, simple branched tubular; *e*, simple alveolus; *f*, *g*, simple branched acinous. The secretory portions are black.

Classification of Multicellular Glands. It is not possible to give a satisfactory classification of the multicellular epithelial glands. Functional qualities fail as criteria because in most cases the chemical character of the secretion has not been determined. Of the morphological qualities, the outer form of the terminal portions or the size and form of their lumen are usually considered as important. Three types of terminal portions occur. If the glandular cells line the wall of a cylindrical, branched or nonbranched, blind cavity, it is a *tubule* (Fig. 256 *a*); if they line a spherical or oval sac with a conspicuous free lumen, it is an *alveolus* (Fig. 256, *e*); and if they line a round or

Simple exocrine glands. The sum of the glandular cavities forms one architectural unit or system. When an excretory duct is absent, the terminal portions open directly on the epithelial surface. If there is an excretory duct, it is not branched and the terminal portions all open directly into it. According to the form of the terminal portions, the following types are distinguished in man: simple tubular, simple coiled tubular, simple branched tubular, and simple acinous or alveolar.

1. *Simple Tubular Glands.* There is no excretory duct and the terminal portion is a straight, blindly ending tubule; it opens directly on the epithelial surface (Fig. 256, *a*). Such are the glands (crypts) of Lieberkuhn in the intestine.

2. *Simple Coiled Tubular Glands.* The terminal

found to exist between particular glands, have given rise to a host of theories and speculations as to the rôle of these glands comes separated from the epithelial surface and its cells form a compact mass which is thoroughly penetrated by a dense

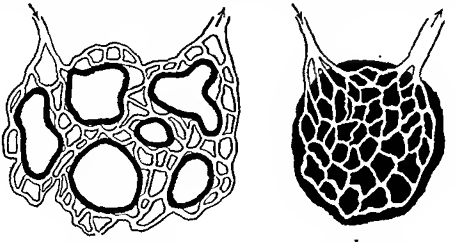

Fig. 258 Diagrams of endocrine glands. *a*, Gland composed of Irregular sacs (heavy black lines) surrounded by connective tissue and blood vessels This type Includes the thyroid and ovary. *b*, In this type the epithelium (black) has no lumen and is penetrated by dense networks of blood vessels (white). To this group belong most of the endocrine glands: suprarenals, islands of Langerhans, parathyroids, hypophysis, corpus luteum, etc.

in the bodily economy. Great advances in the knowledge of the *specific functions* of many of the endocrine glands have resulted from physiological and biochemical investigations, and the pathology of certain diseases in man.

The generally recognized glands of internal secretion are the adrenal, hypophysis, thyroid, parathyroid, islets of Langerhans in the pancreas and portions of the gonads. The thymus, pineal body and the paraganglia are included in this group, although the evidence for their endocrine nature is not clear. The endocrine activities of the gonads, the islets of Langerhans and the gastro-intestinal tract will be discussed in the chapters on the sex glands, pancreas, stomach and duodenum.

As these glands develop in the embryo, the connection with the surface epithelium is lost. In some cases the gland consists of sacs lined with epithelium and surrounded by connective tissue (thyroid. Fig. 258, *a*). In most cases, the invagination of the epithelium loses its lumen or is solid from the very beginning. It becomes

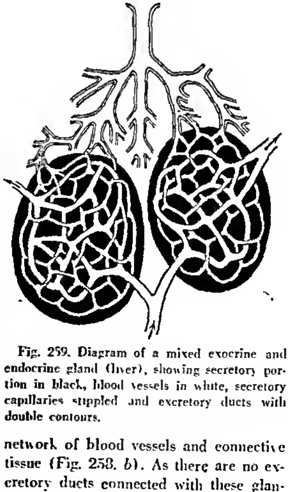

Fig. 259. Diagram of a mixed exocrine and endocrine gland (liver), showing secretory portion in black, blood vessels in white, secretory capillaries stippled and excretory ducts with double contours.

network of blood vessels and connective tissue (Fig. 258. *b*). As there are no excretory ducts connected with these glandular cells, all substances eliminated by them enter the general circulation.

substances in a given tissue and their transfer to the blood stream which carries them to other tissues and cells upon which they act.

All organs give up to the blood or lymph chemical compounds which they have elaborated as a part of their metabolism; sometimes these are peculiar to particular glands or cells. Claude Bernard discovered that the liver synthesizes glycogen from the glucose brought to it, stores this material, and then gives it up to the blood passing through it as glucose. He called this an internal secretory function of the liver in contrast to the external secretion of the bile. Since then the idea of internal or endocrine secretion has become limited by some to mean the elaboration of characteristic chemical compounds (*hormones*) which depress, activate or maintain the function of particular cells or tissues or the whole organism. Such authors prefer to speak of the metabolic rather than the endocrine functions of the liver, for instance. An opposed view is held by those who emphasize that most of the cells in the body give off to the blood substances which affect distant organs and cells. It is perhaps best to designate substances which may arise in many different cell types as *parahormones* and to reserve the term *hormone* for those substances which are formed in only one type of cell. This distinction is made not only on the production of a specific product, but also on the specificity of cellular origin. However, with this definition it is difficult to separate the macrophages, because of the antibodies which they are supposed to elaborate, from the group of endocrine glands (p. 98). Relatively minute amounts of hormones produce marked effects on tissues and organs. In certain glands the secretions are potent in dilutions of one part in many millions. Several of the endocrine organs are indispensable for life.

The secretions of the endocrine glands are of two main types. In one group, such as the nervous lobe of the hypophysis and the medulla of the adrenal, exceedingly small amounts of secretion produce intensely dramatic but fleeting pharmacodynamic effects. Most of the other endocrine glands elaborate materials which must be given in relatively large amounts, at least in experiments, over long periods of time to be effective. It must be noted, however, that actions quite as specific, dramatic, and diversified as any so far described for the hormones are carried out in biological systems by distinctly different classes of substances, as vitamins, the biologically rare metals, and most enzymes.

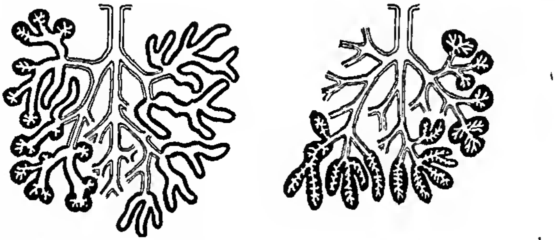

Fig. 257. Diagram of compound exocrine glands. Secretory portions black; ducts double contoured.

The striking effects caused by certain of the hormones, as well as the relationships and interdependencies which have been

Chapter XIV

THE ENDOCRINE GLANDS

HYPOPHYSIS CEREBRI

THE hypophysis is one of the most important organs in the body. It measures about 1 cm. in length, 1 to 1.5 cm. in width, and about 0.5 cm. in height; it usually weighs about 0.5 gm., but becomes much larger in pregnant women. It is lodged in the sella turcica of the sphenoid bone, where it is surrounded by the dura mater and is attached to the floor of the third ventricle by a thin stalk; it is in close relationship with the optic chiasma. According to Wislocki, the subdural space does not extend around the body of the organ The organ consists of two main parts, an anterior and a posterior, which are separated in young human individuals and in many adult mammals by a thin cleft, a rudiment of the lumen of Rathke's pouch. In adult man it becomes a row of vesicles (Fig. 263). The portion anterior to this cleft is called the *pars distalis* and is capped by the small *pars tuberalis.* Posterior to the cleft are the *pars intermedia* and the *pars nervosa.* Thus, the pars intermedia separates the pars distalis (the anterior lobe proper) from the pars nervosa. The relationships are shown in Fig. 260.

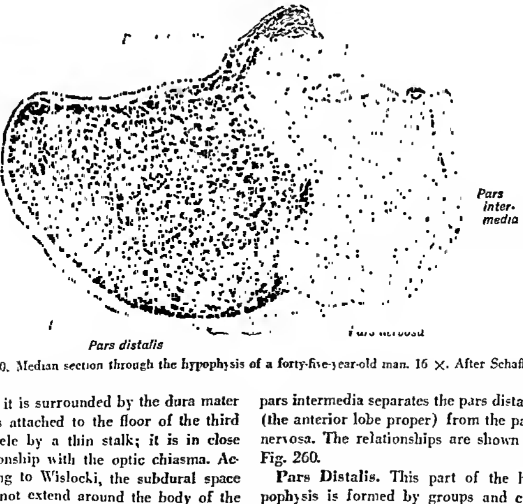

Fig. 260. Median section through the hypophysis of a forty-five-year-old man. 16 ×. After Schaffer.

Pars Distalis. This part of the hypophysis is formed by groups and columns of epithelial cells supported in a delicate reticular connective tissue. Between them are dilated sinusoids lined by

Mixed Exocrine and Endocrine Glands. The liver of the mammals belongs to this group. This gland arises in the embryo as a compound tubular gland whose branching terminal portions anastomose with one another in the form of a net. In this condition the liver remains in the adult lower vertebrates. In adult mammals the tubular cell cords form a compact mass penetrated by blood vessels and by an elaborate system of secretory capillaries which continue into a system of excretory ducts (Fig. 259). The latter serve for the elimination of bile, the external secretion, into the intestine. The blood vessels which partially surround each glandular liver cell receive its endocrine secretions.

In other mixed glands, as the testis and pancreas, the external and internal secretions are formed in separate types of cells. In the liver, however, only one kind of epithelial cell is present.

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and basophil types are usually distinguished. This difference in staining reaction is due to differences in the granules in the two cell types. It is perhaps better to call them alpha and beta cells, for the so-called *acidophil cells* also stain with basic dyes (see Fig. 261, in which the "acidophil" cells are stained with safranine, a basic dye). The alpha cells form about 37 per cent and the beta cells about 11 per cent of the epithelium. The *beta cells* are usually slightly larger than the alpha cells.

all the cell types although some are vesicular while others are smaller and contain heavier chromatin granules. The mitochondria are usually rod-shaped or filamentous. There are two kinds of Golgi nets in the chromophils and the same two kinds in the chromophobes in guinea pigs. This is not the case in man.

Most of the chromophobe cells, the so-called *chief* or *principal* or *reserve cells*, have relatively small amounts of cytoplasm and are connected by transition forms with the chromophil cells. It is also

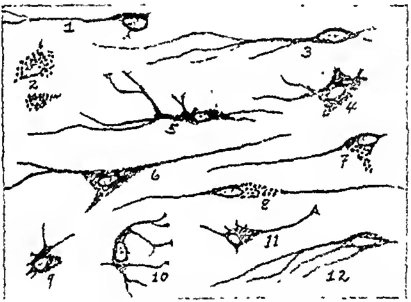

Fig. 262. Isolated specific cells of the pars nervosa of the hypophysis of an ox, stained differentially with Penfield's silver carbonate method Several of the processes (cells *5, 10, 11*) have terminal expansions. Cells *2, 7, 8* contain pigment granules. All of these cells are neuroglia cells specific for the pars nervosa About 700 ×. After Bucy.

The *alpha granules* are spherical, stain pink with eosin, and are larger than the beta granules, which stain with hematoxylin and usually fill the cell completely. It is claimed that the granules tend to accumulate most on the sides of the cells nearest the sinuses and that they contain the precursors of the secretion. In the rabbit and cat another type of acidophil cell has been described. Its granules stain red with the azocarmine of the Mallory-azan method, while the ordinary acidophil cells stain with the orange dye of this mixture. The nuclei are about the same in

possible that some of the chromophobe cells are chromophil cells which have lost their granules. The alpha cells are distributed more or less regularly throughout the pars distalis while the chromophobe cells are more numerous near the stalk.

Pars Tuberalis. The pars tuberalis has not been studied very thoroughly in man, but in the cat and dog it is composed of columns of cells separated by sinuses so that in general it resembles the structure of the pars distalis. The cells of the pars tuberalis, however, do not contain granules, but may form irregular cavities

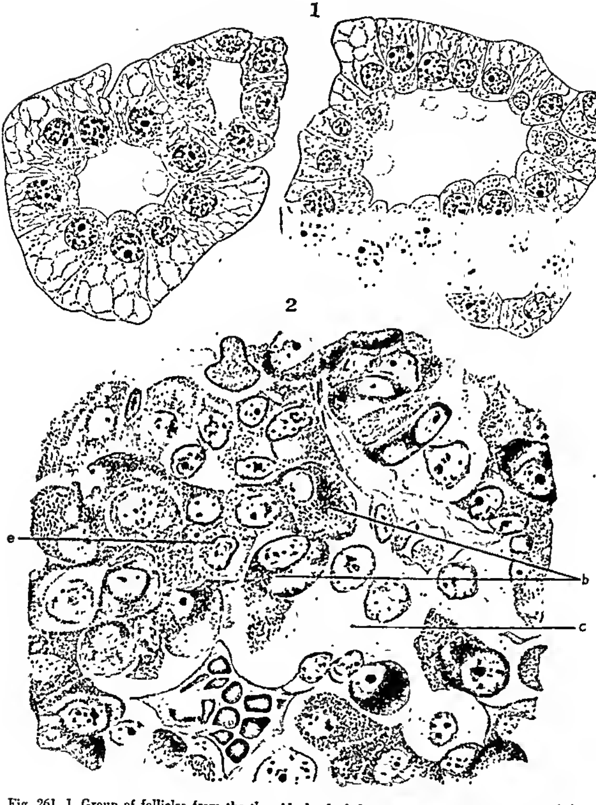

Fig. 261. 1, Group of follicles from the thyroid gland of the opossum. In the outer poles of the cells is a differentially stained material similar to the intrafollicular colloid. Stained with brasilin-wasserblau. 1050 ×. After Bensley.

2, Pars distalis of the hypophysis of a pig; *e*, alpha cell; *b*, beta cells; *c*, chromophobe cell Safranine-acid violet stain. After Maurer and Lewis.

macrophages. Occasionally, the epithelial cells form acinus-like structures with lumens. About half of the epithelial cells are chromophil, the others are chromophobe (Fig. 261, *e*, *b*, *c*).

Among the chromophil cells, acidophil

in the pars nervosa connected with thin nerve fibers. The origin, nature, and significance of these hyaline bodies are unknown. It is claimed that they persist after the potent hormones have been extracted from the pars nervosa. The belief that these hormones are elaborated in the cells of the pars intermedia should be discarded, for in those animals in which there is a complete anatomical separation of the pars nervosa from the pars distalis and in which a pars intermedia is lacking (whale, manatee, armadillo, chicken), the characteristic pressor, antidiuretic and oxytocic hormones have been extracted only from the pars nervosa. In these animals, the melanophore-expanding hormone, *intermedin*, which is usually ascribed to the pars intermedia, arises in the para distalis.

Basophil cells, smaller and less vacuolated than those of the pars distalis, can be found with regularity in the human pars nervosa; they increase in number with age. In very young children, branching glands lined by a low columnar epithelium have also been found in the pars nervosa. These seem to disappear with age.

Pars Intermedia. This part of the gland is quite small in man and its limits are not sharply demarcated (Fig. 263). It consists of a few rows of basophil granular cells which are usually slightly smaller than those of the pars distalis. In addition, nongranular cells are arranged in follicles whose lumen is filled with a hyaline material, much like the colloid of the thyroid gland in appearance but lacking the iodine of the latter. The basophil cells of the pars intermedia merge with those of the pars distalis and continue for surprising distances into the pars nervosa in man (Rasmussen).

Blood Vessels. The pars distalis and the pars tuberalis have a rich blood supply; the pars nervosa is less vascular and the pars intermedia still less so. The arterial supply is mainly from the inferior hypophyseal branches of the internal carotid and from the superior hypophyseal arteries arising from the internal carotid and the circle of Willis. The venous drainage is mainly into the cavernous sinus. According to Wislocki and King, the sinuses of the pars distalis, in addition to their arterial blood, also receive venous drainage of the hypophyseal stem. These authors did not confirm the finding of Popa and Fielding of a portal system of veins passing up the hypophyseal stalk into the hypothalamus.

Nerves. The pars distalis receives unmyelinated fibers from the carotid plexus. These fibers end between the epithelial cells; their nature and function are unknown. A thick bundle of unmyelinated fibers which originate in the vicinity of the supra optic nucleus above the optic chiasma descends in the infundibular stalk to spread throughout the pars nervosa, and in smaller number in the pars intermedia also. These fibers have branched endings which may terminate in large end-bulbs among the specific elements of this part of the hypophysis.

Histogenetic Remarks. The tissue of the hypophysis arises from two widely separated sources; one of these is an evagination of the ectoderm of the primitive buccal cavity and extends as the pouch of Rathke toward the embryonic brain; this constitutes the *pars buccalis.* Kingsbury et al. maintain that this is not an active outgrowth and that the hypophyseal part of the ectoderm and infundibular part of the brain are originally close together and this relation is retained. The most advanced part of this outgrowth comes in contact with a ventral evagination of the diencephalon, the former becoming the pars intermedia, the latter, the pars nervosa The remnants of the pars buccalis divide into two lateral lobes (in embryos of 10.5 mm.) and a large anterior portion. The latter is transformed into the anterior or distal lobe of the definitive gland. In 45 mm. embryos, the two lateral lobes of the pars buccalis fuse at the midline to form the pars tuberalis and begin to grow forward; this portion, later, grows backward, surrounds the infundibulum, and spreads for a short distance under the tuber cinereum. The pars buccalis in man normally becomes completely separated from the buccal ectoderm. The pars nervosa develops as a downward outpouching of the floor of the diencephalon. Although in some animals, it is more or less completely surrounded by the pars buccalis, this is not the case in man. The pars nervosa remains connected, by a stalk of the infundibulum, with the floor of the third ventricle; its cavity disappears in man, but is retained in the cat. The following schema illus-

in which a colloid material, unlike that of the thyroid gland, accumulates. These cavities are not formed until after birth. The pars tuberalis also contains scattered nests of squamous epithelial cells, perhaps rudiments of the buccal ectoderm.

Pars Nervosa. As this portion of the hypophysis arises from the floor of the third ventricle of the brain in early embryonic stages, it contains a cavity which is continuous with that of the ventricle. In man and many other mammals, the cavity becomes obliterated, although it persists in the adult cat. In man the pars nervosa (Fig. 260) is composed almost entirely of fusiform and irregularly shaped cells which are provided with delicate processes (Fig. 262). According to Gersh (1937), these polymorphous cells when living contain large numbers of refractile granules and droplets; after fixation in Maximow's liquid (Zenker-formol-osmic), they are blackened with the osmic acid. In dehydrated rats these cells become larger. In animals delivering young and in pigeons while laying eggs, the cells become larger and more numerous. In addition to these droplet-containing cells there seem to be undifferentiated precursors from which they develop.

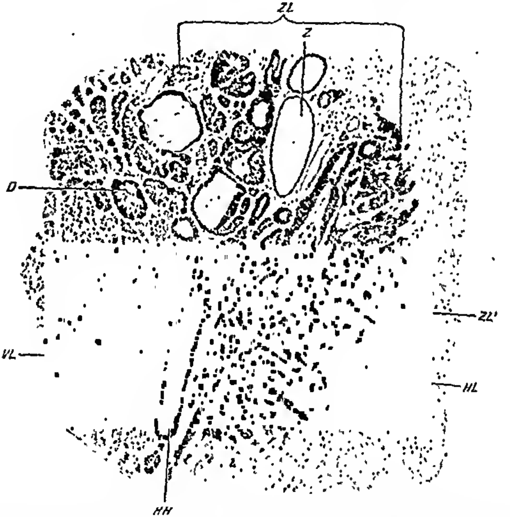

Fig. 263. Midportion of hypophysis of a forty-five-year-old human in median section. *D*, Glandular cord with dark basophil and pale oxyphil cells. *HH*, vestige of hypophyseal cavity; *HL*, pars nervosa; *VL*, pars distalis with chromophobe cells. *Z*, cyst with colloid-like content; *ZL*, pars intermedia; *ZL'*, extension of pars intermedia into the nervous portion. 80 ×. After Schaffer.

The cells of this portion of the gland are of ependymal origin; they are not modified nerve cells and never contain Nissl substance. Hyaline bodies are found

cle of the uterus and raises the blood sugar. These substances have not been demonstrated with certainty in the blood stream, perhaps because of their extreme potency and their consequent presence in very high dilution. In any event, their rôle in the normal bodily economy is yet to be demonstrated.

Injury to the supra-optic tract leads to a degeneration of the cells in the pars nervosa and to a condition simulating the disease called *diabetes insipidus,* in which there is extreme polydipsia and polyuria. The polyuria is thought by some to be due to interference with reabsorption of water by the tubules of the kidney. This condition may be alleviated temporarily and sometimes permanently by the administration of extracts of the pars nervosa. Extracts from such degenerating glands show a decreased or even no content of the usually extractible hormones of this gland (Ranson et al). The condition does not arise after complete hypophysectomy and can be alleviated by removal of the anterior lobe, perhaps affecting water balance through the adrenal cortex.

It seems that the only hormone produced by the intermediate lobe is the melanophore-expanding principle.

Many other interrelationships have been claimed for hypophyseal extracts and the other endocrines. The purification of the various hormones of the hypophysis is clarifying much that has been obscure and controversial; it is also bringing new problems to light.

THYROID GLAND

The thyroid gland is one of the most important of the endocrine organs. It is situated in the anterior, middle portion of the neck, close to the trachea, and consists of two lateral portions or lobes united by a thin strip, the "isthmus." Sometimes there is an irregular pyramidal lobe extending toward the thyroid cartilage.

The external connective tissue capsule of the gland continues into the surrounding cervical fascia; it is connected by loose connective tissue with the dense connective tissue which adheres intimately to the organ. This separation of the capsule into two layers permits the organ to be removed relatively easily.

Parenchyma. The parenchyma consists of anastomosing plates which contain the thyroid *follicles* and are separated from one another by irregular masses of connective tissue (Fig. 264). The epithelium-lined, irregularly spherical follicles are separated from one another by a reticular connective tissue which carries the blood, lymph, and nerve supply to them. Although the epithelium lining them may be exceedingly pedunculated in certain pathological conditions, the external form of the follicles is usually roughly spherical. Most of the evidence shows that new follicles are not formed after puberty, and that the appearances described as buds are irregularly twisted follicles which have entered the plane of the section at more than one point (Fig. 264).

The lumen of the follicles is filled with a characteristic material, called *colloid,* containing large amounts of iodine (Fig. 266). In the fresh state, this material is clear and viscid; when fixed it stains deeply with acid dyes (Fig. 271), although with certain stains it may show various grades of basophilia. Nucleoprotein has not been demonstrated in the colloid on examination with ultraviolet light. On the basis of the basophilia of the colloid and removal of the basophilic substance with ribonuclease it is thought that nucleoprotein is probably present. Since the follicles with basophil colloid were found to contain less I^{131} than those with acidophil colloid, it has been suggested that the turnover and excretion of iodine takes place more readily in the follicles with the basophil colloid. A few desqua-

trates the origin of the various parts of the hypophysis:

Pars buccalis { pars tuberalis
pars distalis—anterior lobe
pars intermedia }
pars nervosa } posterior lobe

Histophysiologic Remarks. The onterior lobe secretes a hormone which stimulates the growth of the body, especially of the skeleton. Certain tumors of the pars distalis cause gigantism in children due to an increase in length of the bones, while in adults, the bones become heavier and thicker ond the condition is called acromegoly.

When the hypophysis is removed from young animols, growth of the body ceoses. If such animols ore injected with the *growth-promoting hormone* of the pars distalis growth proceeds ogain; this hormone is probably elaborated by the olpha cells. Simultoneous odministration of thyroid extract augments the oction of the growth hormone, but simultaneous administration of the pituitary odrenocorticotrophic hormone inhibits its action. Other hormones have been separated from the pars distolis. The two *gonadotrophic hormones* produce profound effects on the gonads: the *follicle-stimulating hormone* stimulates spermatogenesis and an increase in number and size of the ovarian follicles, while the *luteinizing hormone* causes luteinization of those follicles and stimulates the interstitial cells of the testis. Much of the work with these gonadotrophic hormones has been done with rats; other species are not affected in exactly the same way by these extracts. The injection of large amounts of sex hormones depresses the formation of gonadotrophic hormones, while castration causes their storage in the hypophysis. There is much evidence that the beta cells form the follicle-stimulating hormone. Dawson has correlated the carminophil cells in the cat (Friedgood and Dawson) with the lactogenic hormone.

The *thyrotrophic* hormone stimulates the growth and secretion of the thyroid epithelium. In hypophysectomized animals the thyroid gland becomes atrophic. Injection of the *adrenocorticotrophic* hormone couses hypertrophy of the glomerular ond fascicular zones of the adrenal cortex, and an increase in the lipid content of their cells, while hypophysectomy is followed by otrophy of the adrenal cortex. The ovarion hormones apporently stimulate the formation of a mammogenic duct hormone in the hypophysis. The *lactogenic* hormone initiates lactation ofter the mammary gland has hypertrophied. Hypophysectomy abolishes lactation. There ore evidences of the pars distalis affecting protein, fat, and carbohydrote metobolism. Hypophysectomy ond injuries to the tuber cinereum counteroct the hyperglycemia of pancreatic diabetes in dogs.

The onterior lobe becomes much larger during pregnoncy, due mainly to an increase in the number of chromophobe cells. After castrotion the beto cells increase in number and become vacuolated while the olpha cells become mucb less numerous. An increase in the number of beta cells has been described in the woodchuck after hibernation. The administration of thyroid substance, of anterior lobe extracts and of epinephrine is said to increase the number of alpha cells, while iodides are believed to reduce the number of both alpha and beta cells. Removal of the thyroid gland causes an increase in the number of chromophobe cells and a decrease in the number of alpha cells. In cultures of the hypophysis, the alpha cells degenerate quickly while the chromophobe cells live longer.

Extracts of the pars nervosa have marked pharmacodynamic actions. From these extracts two fractions have been isolated: *pitressin,* which raises blood pressure and is markedly antidiuretic, and *pitocin,* which contracts the smooth mus-

cle of the uterus and raises the blood sugar. These substances have not been demonstrated with certainty in the blood stream, perhaps because of their extreme potency and their consequent presence in very high dilution. In any event, their rôle in the normal bodily economy is yet to be demonstrated.

Injury to the supra-optic tract leads to a degeneration of the cells in the pars nervosa and to a condition simulating the disease called *diabetes insipidus,* in which there is extreme polydipsia and polyuria. The polyuria is thought by some to be due to interference with reabsorption of water by the tubules of the kidney. This condition may be alleviated temporarily and sometimes permanently by the administration of extracts of the pars nervosa. Extracts from such degenerating glands show a decreased or even no content of the usually extractible hormones of this gland (Ranson et al). The condition does not arise after complete hypophysectomy and can be alleviated by removal of the anterior lobe, perhaps affecting water balance through the adrenal cortex.

It seems that the only hormone produced by the intermediate lobe is the melanophore-expanding principle.

Many other interrelationships have been claimed for hypophyseal extracts and the other endocrines. The purification of the various hormones of the hypophysis is clarifying much that has been obscure and controversial; it is also bringing new problems to light.

THYROID GLAND

The thyroid gland is one of the most important of the endocrine organs. It is situated in the anterior, middle portion of the neck, close to the trachea, and consists of two lateral portions or lobes united by a thin strip, the "isthmus." Sometimes there is an irregular pyramidal lobe extending toward the thyroid cartilage.

The external connective tissue capsule of the gland continues into the surrounding cervical fascia; it is connected by loose connective tissue with the dense connective tissue which adheres intimately to the organ. This separation of the capsule into two layers permits the organ to be removed relatively easily.

Parenchyma. The parenchyma consists of anastomosing plates which contain the thyroid *follicles* and are separated from one another by irregular masses of connective tissue (Fig. 264). The epithelium-lined, irregularly spherical follicles are separated from one another by a reticular connective tissue which carries the blood, lymph, and nerve supply to them. Although the epithelium lining them may be exceedingly pedunculated in certain pathological conditions, the external form of the follicles is usually roughly spherical. Most of the evidence shows that new follicles are not formed after puberty, and that the appearances described as buds are irregularly twisted follicles which have entered the plane of the section at more than one point (Fig. 264).

The lumen of the follicles is filled with a characteristic material, called *colloid,* containing large amounts of iodine (Fig. 266). In the fresh state, this material is clear and viscid; when fixed it stains deeply with acid dyes (Fig. 271), although with certain stains it may show various grades of basophilia. Nucleoprotein has not been demonstrated in the colloid on examination with ultraviolet light. On the basis of the basophilia of the colloid and removal of the basophilic substance with ribonuclease it is thought that nucleoprotein is probably present. Since the follicles with basophil colloid were found to contain less I^{131} than those with acidophil colloid, it has been suggested that the turnover and excretion of iodine takes place more readily in the follicles with the basophil colloid. A few desqua-

mated cells may be found in it, and in sections it is frequently separated from the epithelium surrounding the lumen by large and small vacuoles. This appearance is usually a fixation artefact for the vacuoles are rarely present in normal living follicles and are absent after fixation by the Altmann - Gersh freezing - drying method.

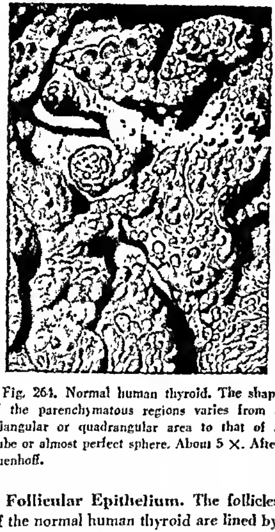

Fig. 264. Normal human thyroid. The shape of the parenchymatous regions varies from a triangular or quadrangular area to that of a cube or almost perfect sphere. About 5 ×. After Rienhoff.

Follicular Epithelium. The follicles of the normal human thyroid are lined by a low cuboidal epithelium. As there is no basement membrane, the cells rest on the interfollicular, reticular connective tissue. and thus are in close contact with its network of blood and lymphatic capillaries and nerves (Fig. 265). The epithelium of the gland shows great variations in size and arrangement, which depend on age sex, season of the year and diet. as well as on certain pathologic processes involving the gland. In general, it is believed that the epithelium becomes very low when the gland is underactive and very tall and folded when the organ is overactive. There are many cases, however, in which the condition of the epithelium does not reflect the activity of the gland so that a determination of the degree of functional activity of the organ cannot be made with certainty on the basis of a histologic examination alone.

The follicles of the thyroid gland are lined by a single type of epithelial cell. The so-called *colloid cells* (described by Langendorff) are in all probability dead or dying cells, such as have been described in many kinds of epithelial membranes. In the cells of the thyroid gland, inclusions, presumably of secretory nature, of the following sorts have been described: (1) Round droplets of colloid first described by Langendorff; (2) clear vacuoles with no recognizable organic content—described by Anderson and connected by him with the vacuoles seen in the colloid within the follicles; (3) the basal, dilute colloid of Bensley (Fig. 261. *1*); (4) minute granules, stainable in the living cell with neutral red (described by Uhlenhuth). The rôle of these several structures in the secretory history of the thyroid gland cell is still under discussion.

It has been claimed that the basophilia of the follicular epithelium with the eosin-methylene blue stain is an indicator of the presence of ribose-nucleoprotein.

The mitochondria in the epithelium of the normal human thyroid are usually short, thin, and rod-shaped (Fig. 265). Most of them are located between the nucleus and the lumen of the follicle. The Golgi net usually is toward the lumen. The claim that the occurrence of the net between the nucleus and the basal (proximal) part of the cell is an indication of secretion toward the blood vessels, has not been substantiated.

A centrosome is located in the distal portion of the cell body near the lumen.

Dividing cells are rare. The epithelium contains fine terminal bars.

Interfollicular Cells. Large and small groups of interfollicular epithelial cells without a distinct lumen have been described repeatedly in the thyroid gland and were considered to be fetal rests. Some of them at least are merely tangentially cut portions of follicular walls. The migration of argyrophil "parafollicular" cells from the follicles into the connective tissue between the follicles has been described in the dog and rabbit.

A few lymphocytes and macrophages are normally present in the interfollicular connective tissue. In certain pathologic conditions, lymphatic tissue, both diffuse and in nodules, may develop in the stroma of the thyroid. Occasionally small bits of thymic tissue and, more frequently, portions of parathyroid may be found included in the thyroid gland.

Blood Vessels. The thyroid gland has an unusually rich blood supply, even for an endocrine organ. It is nourished by the superior thyroid arteries (branches of the external carotids) and the inferior thyroid arteries (arising from the subclavians). These vessels ramify over the surfaces of the gland and then penetrate its substance; they run in the connective tissue partitions between the bands of thyroid parenchyma and send smaller branches to form a dense basketwork over the surface of each follicle (Fig. 265). The blood from these networks is collected into veins which also run in the interfollicular connective tissue. The veins of the thyroid gland are the superior and middle thyroid veins, usually emptying into the internal jugular vein, and the inferior thyroid veins which communicate with the left innominate vein.

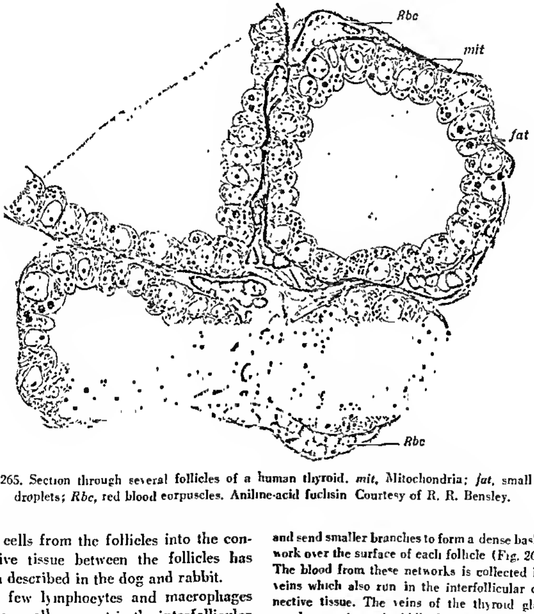

Fig. 265. Section through several follicles of a human thyroid. *mit*, Mitochondria; *fat*, small fat droplets; *Rbc*, red blood corpuscles. Aniline-acid fuchsin Courtesy of R. R. Bensley.

Lymphatics. The thyroid gland is richly supplied with lymphatic vessels. These arise in the interfollicular connective tissue, whence they con-

tinue into larger trunks, forming a dense plexus beneath the capsule. These usually drain into the low cervical lymph nodes, but in certain animals they are described as emptying directly into the thoracic or right lymphatic ducts before they enter the jugular or subclavian veins. Thyroglobulin has been found in the efferent lymphatics.

Nerves. The thyroid gland receives postganglionic fibers from the superior and middle cervical ganglia. These sympathetic nerves enter the gland with the blood vessels, in whose adventitia they form numerous plexuses. Many of the fibers from these plexuses are of purely vasomotor nature; others end in networks and in simple terminals about the epithelium of the follicles. The secretory nature of these fibers has not been proved. The thyroid gland also receives a few fibers from the superior and recurrent laryngeal nerves. The intraglandular course and the nature of these fibers are unknown.

Regeneration. The thyroid gland regenerates rapidly after surgical reduction if iodine is withheld from the diet, but will not regenerate if desiccated thyroid substance is administered. The gland may be transplanted readily into the same animal, particularly if it is suffering with a thyroid deficiency. Pure cultures of embryonic chick thyroid epithelium have been kept alive for some months.

Histogenesis. In man the primordium of the thyroid gland arises very early (embryos of 1.37 mm.) as a medial ventral outgrowth of the entoderm, cranial to that of the trachea. The foramen cecum at the base of the tongue of the adult is the point from which the diverticulum arose in the embryo. In man there does not seem to be a contribution to the thyroid from the fourth branchial pouch.

At first the primordium is a hollow tube which grows caudally and thickens at its end. The connection between the tongue and the thyroid gland usually disappears (embryos of 4 to 7 mm.), but sometimes it persists, either as the *thyroglossal duct* or as an irregular mass of thyroid tissue, usually eccentrically located, which is called the *pyramidal lobe*. The primordium then becomes a solid mass of epithelium, which later splits into ramifying plates and cords of epithelium. Hollows arise within these cords; these are the primary follicles and are always empty. They later fuse with one another and are invaded by mesenchyme. The walls of these hollows are two cells thick. Then the follicles of the mature organ arise by the repeated constriction of these plates into roughly spherical structures, in which a cavity lined by a single layer of epithelial cells develops; these definitive follicles are surrounded by mesenchyme (embryos of 24 mm.). Colloid may be present before birth, but does not become an important constituent of the follicles until after birth. In the fetal pig (gestation period 114 days) inorganic iodine is first found at 46 to 50 days, di-iodotyrosine at 52 days and colloid and more di-iodotyrosine at 60 days. With the

Fig. 266. Low power photomicrograph of autoradiograph of thyroid gland of rat previously injected with I^{131}. The blackened areas represent sites of deposition of the radioactive material. There is great variability in the content of the isotope in the several follicles. In a few places the epithelium is blackened. Courtesy of C. P. Leblond, D. Findlay and S. Gross.

aid of I^{131} it has been found that the storage of iodine begins in the rat embryo at 18 to 19 days, at which time follicles with lumens appear.

Histophysiologic Remarks. The thyroid gland is characterized by its high content of iodine. The extractable hormone of the thyroid gland is called *iodothyroglobulin;* from it *thyroxine* has been isolated. Thyroxine has most of the pharmacologic properties of iodothyroglobulin and is a derivative of di-iodotyrosine. Whether the thyroid gland elaborates other substances is unknown.

The details of secretion in the thyroid gland are still obscure, although some advances toward the solution of this problem have been made in recent years. Studies of the gland in the living animal and after fixation by the freezing-drying method and examination with ultraviolet light of wave lengths specific for thyroglobulin all indicate that secretion is normally toward the lumen of the follicle from which the colloid is later reabsorbed. When the gland is greatly stimulated the secretion is chiefly toward the blood stream. It has been shown with the aid of I^{131} that this element is rapidly concentrated in the thyroid gland and that all of the follicles are not in the same state of activity at a given time (see Fig. 266).

A proteolytic enzyme of the *cathepsin* type has been found in the colloid of the follicles. It is responsible for the hydrolysis of the protein which makes its reabsorption possible. *Hyaluronidase* (the spreading factor) is also present; it affects the viscosity of the colloid and varies in activity with the state of the gland. The viscosity of the colloid diminishes after injection of the thyrotrophic hypophyseal hormone and after feeding potassium iodide.

Perhaps the most striking effect of the thyroid secretion is its control over the metabolic rate of the entire body. When a deficiency of this secretion is present, the metabolic rate is below normal, and when it is overactive an increase in this rate takes place. Disturbances in thyroid activity are manifested by two spontaneous conditions in man; these are known as *simple goiter* and *myxedema.* Effects similar to myxedema are brought about by the extirpation of the gland. The simple goiter is characterized by the development of a large thyroid gland in which the follicles contain much colloid and are usually lined by flattened epithelium; to some extent it may be prevented by providing the organism with a sufficient intake of iodine in the food. The basal metabolic rate is unchanged in simple goiter and there are no symptoms of thyroid deficiency. The symptoms of myxedema, which occur congenitally (cretinism) and in adults, may be removed through the timely administration of the dried substance of the thyroid gland.

In *exophthalmic goiter* the thyroid gland is markedly overactive. When such a gland is examined microscopically, the follicular lumens are found to contain little or no colloid and the epithelium is usually quite tall and redundant, so that numerous folds extend into the lumens. The alleviation of the symptoms in this condition, including the increased basal metabolic rate, may be accomplished temporarily by the administration of iodine. *Thiouracil* and other thio-compounds stop the production of thyroid hormone, although the epithelium may be markedly hyperplastic. These drugs do not affect the utilization of thyroid hormone by the body. The primary seat of exophthalmic goiter may possibly be in the pars distalis of the hypophysis, although the condition is often cured by removal of a great part of the thyroid. The administration of the thyrotrophic hormone of the pars distalis of the hypophysis to guinea pigs produces temporarily many of the symptoms of exophthalmic goiter, including the exophthalmus. In these animals the thyroid

shows a marked hyperplasia and loss of colloid.

The thyroid secretion affects other endocrine glands. Its removal produces hypertrophy of the pars distalis of the hypophysis, with disappearance of the eosinophil granules. It also affects the gonads, although the evidence for this is conflicting. The gland hypertrophies during menstruation and pregnancy. Thyroidectomy hastens involution of the thymus; the latter becomes hypertrophied in exophthalmic goiter. Feeding thyroid extract to thyroidectomized rabbits decreases the hypoglycemia resulting from insulin. Thyroglobulin is believed to stimulate the chromaffin system. The thyroid gland becomes markedly hypoplastic in rats suffering from a deficiency of vitamin E.

THE PARATHYROID GLANDS

In man there are usually four or five parathyroid glands, small, yellow-brown, oval bodies intimately connected with the posterior surface of the thyroid gland. In the adult, each body on the average weighs 0.035 gm. and measures 6 to 7 mm. by 3 to 4 mm. by 1.5 to 2 mm. One of them is located on the posterolateral and another on the inner surface of each of the two lateral lobes of the thyroid gland; they are usually in the connective tissue capsule of this gland and only rarely embedded in it. In addition, aberrant parathyroids occur not infrequently in the thyroid or thymus, or in the connective tissue between them. In all normal cases, the tissue of the parathyroids is separated by a connective tissue capsule from the contiguous tissues (Fig. 267).

Fig. 267. Photomicrograph of a section of the thyroid and parathyroid glands of *Macacus rhesus*. 80 ×.

The parathyroid glands are composed of densely packed groups of cells, sometimes arranged in cords. Between the cells is a framework of reticular fibers and a richly anastomosing network of sinusoidal capillaries. Two main types of epithelial cells have been described in it: principal cells and oxyphil cells (Fig. 268). The most important of these are probably the principal cells. Their pale clear cytoplasm practically never contains granules; these cells have a relatively large vesicular nucleus. It is said that these are the only cells found in human parathyroids until about the age of ten years. In some cases

the principal cells form a continuous mass while in other cases they form trabeculae separated by sinuses. Occasionally they may give rise to acini with a colloidal material in their lumen.

The oxyphil cells are somewhat larger than the principal cells and their granular cytoplasm stains intensely with acid dyes; they resemble somewhat the acidophil cells of the hypophysis. The nucleus is small and deeply staining. The oxyphil cells, too, may form continuous masses, consequently no fixed cellular polarity has been determined. Glycogen and neutral fat are normally present. Small islands of adipose tissue may accumulate in the stroma, particularly with advancing age.

After extirpation of the thyroid gland as well as with advancing age, colloid may accumulate in what seem to be follicles of parathyroid tissue. This is probably an indication of a degenerative process; the older idea that this showed a re-

Fig 268. Section of a human parathyroid gland. Bielschowsky-Mallory-azan stains. 540 ×. (W. B.)

anastomosing columns, or even acini with colloid. The latter formation is very rare.

Another type of cell, intermediate between the above two types, has been described. It has a fine granular cytoplasm which stains faintly with acid dyes and a nucleus which is smaller and stains darker than that of the clear principal cells. Transitions between these cell types, and the presence of still other cell types have also been described. The cytology of the normal gland should be studied further.

The evidence indicates that the principal cells are the essential elements of the gland. Both they and the oxyphil cells contain mitochondria. The Golgi net occupies a variable position in the cell and lationship to the thyroid gland has been shown to be incorrect.

Vessels and Nerves. The parathyroids receive an abundant blood supply from branches of the superior and inferior thyroid arteries. These vessels enter at the hilus and break up into many branches which run in the septa and form dense networks about the epithelial cells. The sinus-like capillaries are surrounded by a dense network of reticular fibers which directly touch the epithelial cells. There is no basement membrane. The main vein leaves the hilus and empties into nearby veins of the thyroid gland, trachea, or esophagus. A continuous network of dilated veins has been described beneath the capsule in the herbivora and man.

A few unmyelinated nerves from the cervical

sympathetic enter the gland with the artery. They are probably all vasomotor nerves.

Regeneration. The parathyroids have but insignificant powers of regeneration. The glands "take" readily in autotransplants.

Histogenesis. The parathyroid glands develop from thickenings of the third and fourth branchial pouches on each side. The primordium on the third arch is close to the bud of the thymus. This mode of origin is a probable explanation of the frequent occurrence of aberrant parathyroid bodies in or near the thymus.

Histophysiologic Remarks. That the cells of the parathyroid glands elaborate a hormone which is potent is known from clinical and experimental evidence. Extirpation of the glands is followed by *hypoparathyroidism*, characterized by a decrease in the concentration of calcium in the plasma, and *tetany*. The symptoms may be alleviated by administration of calcium, or of an extract of parathyroid glands. A condition of *chronic latent tetany*, with low plasma calcium but without symptoms, may be produced experimentally in dogs.

Tumor or hyperplasia of the parathyroids may lead to *hyperparathyroidism*, associated with high plasma calcium, extensive bone changes (*osteitis fibrosa*, see p. 152) and pathological calcification in soft tissues. Similar effects may be produced in susceptible animals by the administration of toxic doses of *parathyroid extract*. When rats are injected with a large dose of parathyroid extract, the cells of the parathyroid glands shrink and the Golgi apparatus becomes smaller and compact (suggestive of hypofunction). After two weeks the cells have returned to normal size and the Golgi net has become large and irregular—indicative of the normal secretory activity of these cells. Other cytological evidences of secretion have been described by S. H. Bensley.

The parathyroid glands become large in rickets through increase in size and especially in number of their cells. The hypertrophy is greater in low calcium than in low phosphate rickets. The Golgi net is described as undergoing changes which indicate great secretory activity in the cells as compared with resting cells (DeRobertis). Hypertrophy is also observed in nephritis with uremia, and has resulted from the experimental production of renal insufficiency. It is probably a response to a lowered calcium ion concentration in the plasma resulting from phosphate retention. A similar but extreme hypertrophy with hyperfunction occurs in young individuals following loog-continued renal insufficiency and is associated with extensive bone changes (*renal rickets; renal osteitis fibrosa*) similar to those of primary hyperparathyroidism.

The function of the parathyroid glands is closely linked with the behavior of *calcium* in the organism. The maintenance of a physiologically constant concentration of *calcium ions* in the blood plasma appears to be accomplished by regulation, by the parathyroid hormone, of the movement of calcium from bone to blood. The mechanism of the influence of the parathyroids upon *decalcification* is but poorly understood.

There is some evidence that a hormone of the pars distalis of the hypophysis affects parathyroid secretion.

THE ADRENAL GLANDS

The paired adrenal or suprarenal glands of man are roughly triangular, flattened bodies, one at the cranial pole of each kidney. The glands together average about 10 to 12 gm. in the healthy adult, and measure approximately 5 by 3 by less than 1 cm. An indentation on the anterior surface, the hilus, emits the suprarenal vein.

The surface made by cutting through the gland presents a bright yellow cortex in its outer part and a reddish brown zone which abuts against the thin gray medulla.

Cortex. The cells of the cortex are disposed in three vaguely defined layers: an

outer *zona glomerulosa,* a middle *zona fasciculata,* and an inner *zona reticularis.* The cortical cells in these zones differ somewhat in arrangement and structure. The transition from one zone to another is very gradual.

The narrow zona glomerulosa (Fig. 269) just inside the capsule, consists of small columnar cells closely packed in ovoid groups, or in arcs which surmount the straight cell cords of the zona fasciculata. The free edge of each cell in most cases adjoins a capillary. The nuclei stain deeply and the rather *scanty cytoplasm* contains irregular clumps of material that take nuclear stains, in man. There may be a few tiny lipoid droplets in the glomerulosa cells, particularly if the whole cortex

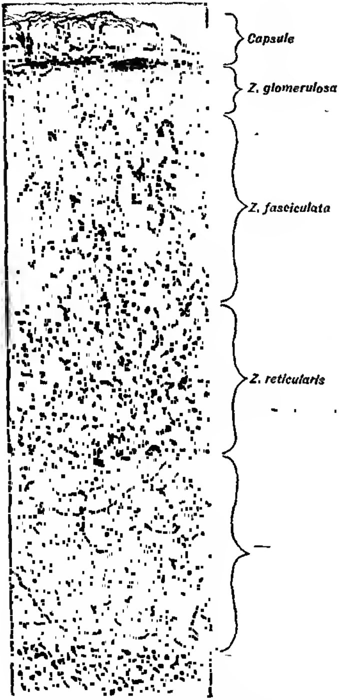

Fig. 269. Section of an adrenal gland of a man. Mallory-azan stain. About 105 ×.

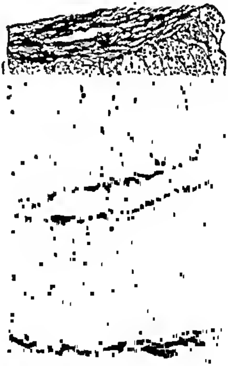

Fig. 270. Section of an adrenal gland of a six months' infant. Mallory-azan stain. About 105 ×.

sympathetic enter the gland with the artery. They are probably all vasomotor nerves.

Regeneration. The parathyroids have but insignificant powers of regeneration. The glands "take" readily in autotransplants.

Histogenesis. The parathyroid glands develop from thickenings of the third and fourth branchial pouches on each side. The primordium on the third arch is close to the bud of the thymus. This mode of origin is a probable explanation of the frequent occurrence of aberrant parathyroid bodies in or near the thymus.

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The surface made by cutting through the gland presents a bright yellow cortex in its outer part and a reddish brown zone which abuts against the thin gray medulla.

Cortex. The cells of the cortex are disposed in three vaguely defined layers: an

row threads between the numerous lipoid droplets scattered throughout the cell; when the lipoid is dissolved the cell has a vacuolated cytoplasm.

With appropriate technic the lipoid droplets are seen to be small and numerous in some cells, larger and fewer in others, but of about equal size in any given cell.

The nuclei are centrally placed in the cells, and often there are two in one cell. They are more vesicular than in the zona glomerulosa. In the outer part, especially in the transition region between the two zones, mitotic figures are frequent (see below).

In the innermost zone of the cortex, cell cords form anastomosing networks, hence the name zona reticularis (Fig. 269). The cells of the outer part of this zone differ little from the cells of the zona fasciculata except for a decreased lipoid content, but near the medulla they gradually merge into two new distinct cell types. These have been called "dark" and "light" cells due to their different staining affinities.

The light cells are larger and have rounded contours, a granular, pale-staining cytoplasm and pale vesicular nuclei. The dark cells are smaller and have a deeply staining homogeneous cytoplasm, and shrunken, hyperchromatic nuclei. The dark cells are rich in lipoid droplets and in clumps of yellow or brownish pigment, while the light cells contain little of either lipoid or pigment. There are also in the zona reticularis a number of cells which appear to be degenerating.

Medulla. The boundary between zona reticularis and medulla is usually irregular in the adult, since long columns of cortical cells project into the medulla. The irregular cells of the medulla are arranged *in rounded groups* or short cords surrounded by the sinusoidal venules. When the tissue is fixed in a fluid containing potassium dichromate very fine brown granules are seen throughout the cells; if chromic acid is employed the cytoplasm stains diffusely brown. This is the "chromaffin" reaction; some believe it is due to oxidation and polymerization of the epinephrine, which is elaborated by the medulla. The epinephrine in the medulla also gives a green color with ferric chloride.

In addition to the chromaffin cells there are frequent, single or grouped, sympathetic ganglion cells in the medulla, whose axons end around the chromaffin cells. The medulla also contains collections of small round cells with deeply staining nuclei and very little cytoplasm. Although similar small round cells, the *sympathochromaffin cells*, in the fetal adrenal are the forerunners of the sympathetic and medullary cells, the small round cells in the adult are probably lymphocytes.

Mitochondria are present in all the cells of the adrenal. In the zona glomerulosa they are frequently long threads, and are found at both ends of the columnar cells. In the zona fasciculata they are round granules. In the light cells of the zona reticularis the mitochondria are very small granules and rods restricted to an area close to the nucleus; in the dark cells they are large and irregular in shape. In the medulla they are small spherical granules scattered throughout the cell.

The Golgi net has been described in the cells of the zona glomerulosa and the medulla of the guinea pig, hedgehog, and bat. In them it is usually between the nucleus and the nearest capillary.

Blood Vessels and Nerves. The arteries to the adrenal gland are usually three in number: the superior suprarenal artery from the inferior phrenic branch of the aorta, the middle direct from the aorta, and the inferior from the renal artery. The smallest arteriolar branches in the capsule empty into the capillaries of the cortex, though some of the larger arterioles may pierce the cortex and go directly to the medulla.

The capsule, a thick layer of dense connective tissue, contains the arteries, a nerve plexus and some collections of sympathetic ganglion cells, and sends fine networks of reticular fibers into the cortex along with the capillaries. The fairly straight capillaries, which traverse the cortex radially from the capsule, empty into irregularly

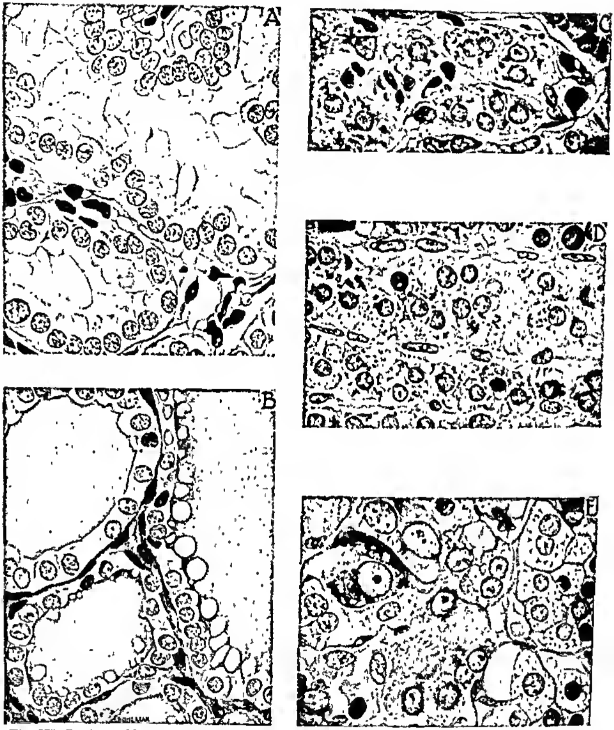

Fig. 271. Sections of human thyroid gland in exophthalmic goiter (A) and colloid goiter (B) from preparations of R. R. Bensley. Note the differences in the height of the epithelium and the amount and depth of staining of the colloid in the two conditions. Hematoxylin and eosin. A and B 570 ×.
C, D, E are sections of the adrenal gland of a man. C is from the zona glomerulosa; D, zona fasciculata, showing narrow columns of cells separated by collapsed blood sinuses; E, corticomedullary junction. The medullary portion to the left contains a large sympathetic ganglion cell with much Nissl substance and several greenish-brown-stained chromaffin cells. The cells of the zona reticularis (to the right) are markedly vacuolated. Hematoxylin-eosin-azure II. C, D, and E 730 ×.

is unusually rich in lipoid; when present, the droplets lie between the nucleus and the side of the cell facing the capillary.

The zona fasciculata (Fig. 269) constitutes the widest portion of the cortex, and consists of polyhedral cells considerably larger than those of the zona glomerulosa. The cytoplasm is reduced to nar-

very minute quantities and is rapidly destroyed in the blood stream. Since it is secreted in greater amounts during nervous excitation, one view is that it makes the body more efficient for necessary responses (emergency theory). Another view is that it is only an excretory product of protein metabolism, since it is probably destroyed before it reaches the arterial blood stream (excretory theory). The medulla is not essential to life.

Cortex. When both adrenals are experimentally removed, the animal dies, owing to loss of the cortical substance. The destruction of all or a large part of the cortex in man (commonly by tuberculosis) leads to the fatal Addison's disease. In experimental animals a fraction of the cortex, when left intact, suffices for the maintenance of life. Completely adrenalectomized animals and patients with Addison's disease exhibit, among other symptoms, increased loss of sodium and of water in the urine, a fall in the concentration of sodium in the plasma, and a rise in plasma potassium. The plasma glucose falls, and the glycogen of the muscles and the liver is reduced in amount. These symptoms can be alleviated, and life prolonged, often indefinitely, by administration of an extract of the cortex. An increased intake of sodium and of water, together with a reduction in the intake of potassium, also has a beneficial effect.

Twenty or more crystalline steroid compounds have been isolated from the adrenal cortex. While these are intimately concerned with the functions of the cortex, no one of them exhibits all of the physiological effects of a crude extract; it is therefore not correct to designate any one, to the exclusion of others, as the adrenal cortical hormone. Of the known steroids, for example, *corticosterone* has a pronounced effect on carbohydrate metabolism while *desoxycorticosterone* exerts its effects chiefly on the metabolism of water and electrolytes. Others of the steroids have estrogenic or androgenic (*adrenosterone*) or progestational activity. There is some evidence that the carbohydrate-regulating principles are secreted by the fasciculata and that the salt-regulating principles are formed in the glomerulosa.

It has been claimed that the adrenocorticotrophic hormone of the anterior pituitary (q.v.) causes the liberation of hormonal substances from the cortex, which produce a disintegration of lymphocytes with a resulting lymphopenia and atrophy of lymphatic tissue, and an increase of beta and gamma globulin of the serum. However, Lawrence and coworkers have not found a similar effect of the adrenal cortex on lymphocytes in cats. The extensive atrophy of lymphatic tissue which results from the action of a variety of noxious agents (part of the "alarm reaction" of Selye) is believed by some to be due to the liberation of adrenal cortical hormone; it does not occur if the adrenal cortex is removed.

The lipoid droplets of the cortex are complex mixtures of cholesterol esters, fatty acids (mainly oleic) and phospholipins. The quantity of sterols increases when the amount in the blood increases, as in pregnancy. The sterols in the cortex disappear rapidly in acute infectious diseases, but do not decrease during starvation, although nearly all the fat disappears from the body. The amount of cholesterol diminishes after excessive muscular activity. Opinions differ as to the exact correlation of the amount of lipoid in and the secretory activity of the cortex. However, the disappearance of the droplets is usually interpreted as an indication of hyperactivity in experimental conditions.

The pigment in the zona reticularis is present only after puberty and increases in amount with age. It is similar to the "lipochrome" pigment found in cardiac muscle and nerve cells in old age.

The cortex has important *interrelationships* with other endocrine organs, espe-

anastomosing, sinusoidal blood spaces in the zona reticularis and medulla. These vessels drain into the central veins which unite and emerge as a single vein. The right suprarenal vein empties into the inferior vena cava, the left into the left renal vein.

The central vein and its larger tributaries are lined by endothelium. They possess abundant smooth muscle fibers arranged mainly in longitudinal bundles. These bundles may make rugose projections into the lumen of the vein.

The lining cells of the sinusoids in the cortex are littoral cells of the macrophage system, much like those lining the sinusoids of the liver (see Fig. 368). They store lithium carmine and in heavily stained animals their number is greatly increased. Between these lining cells and the parenchymatous cells is a dense network of *reticular fibrils.*

Except those about the large veins, lymphatic capillaries have not been demonstrated in the substance of the gland.

The 20 to 30 nerves to each gland come mainly from the celiac sympathetic plexus, in part from the greater splanchnic nerves, and possibly also from the vagus nerves. The numerous nerve fibers end mainly in clawlike terminations around individual cells of the medulla; a few fibers end in relation to cell groups of the cortex, especially in the zona reticularis, but these do not encircle single cells.

Histogenesis. The cortex develops from the celomic mesoderm on the medial side of the wolffian ridge, and the medulla from the ectodermal tissue from which the sympathetic ganglion cells also arise. In the fishes these two tissues remain separated as different organs; in the amphibians, reptiles, and birds the *chromaffin* cells are scattered through the mesodermal tissues; they become aggregated into a definite medulla only in mammals.

In a six weeks' embryo the cortical primordium consists of a rounded bud of cells anterior to the kidney; then strands of sympathochromaffin cells grow ventrally and penetrate the cortical bud on its medial side. At this stage they begin to exhibit the chromaffin reaction, and the cortical cells may already contain fine droplets of lipoids.

The cortex in a three months' fetus, except for a narrow outer zone of small cells with deeply staining nuclei, consists almost entirely of reticulated chains of large granular cells lying between capillaries. The outer zone, from which will be derived the definitive cortex, becomes progressively wider and gradually forms the characteristic straight cell cords. The inner, or *boundary zone* begins to involute soon after birth and is largely gone after the first few weeks. The remainder of the cortex contracts upon the medulla (Fig. 270). Accordingly, for some time the infant's adrenal is smaller than at birth. *Too rapid involution of the boundary zone may lead to fatal* hemorrhage into the adrenals.

The three definitive cortical zones are differentiated by the end of the third year of childhood, the zona reticularis having developed during the third year. The adrenal is as large as the kidney in an eight weeks' embryo, one third as large as the kidney at birth, and one thirtieth as large in the adult.

Regeneration. The cells of the cortex appear to be degenerating and dying continually in the zona reticularis. The débris is removed mainly by the macrophages which are always present. The dark and light cells are probably senescent *cells which are destined to degenerate soon.* Some writers have suggested that the dark and light cells express different stages in a secretory cycle, but the evidence for this is inconclusive. The cells lost in this degenerative process are replaced through mitotic proliferation of the cells in the outer zone of the zona fasciculata. There are several reports that the adrenal cortex may regenerate from the cells of the capsule. This *requires further investigation.* The newly formed cells gradually move inward toward the medulla.

These parallel processes of cell destruction and proliferation are accelerated by acute infectious disease, toxins in the blood stream, prolonged narcosis, etc. The cortical cells are particularly susceptible to injury. From animal experimentation it is found that repair is always effected within two or three weeks if the damage has not been severe enough to lead to scar formation or eventually to death. The medullary cells do not seem to change much, except for the presence of colloid vacuoles containing a protein (Russell's bodies).

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chance to regenerate in those individuals who died as a result of severe infections. At birth the thymus weighs 12 to 15 gm. This increases to about 30 to 40 gm. at puberty, after which it begins to decrease in weight so that at sixty years it weighs only 10 to 15 gm.

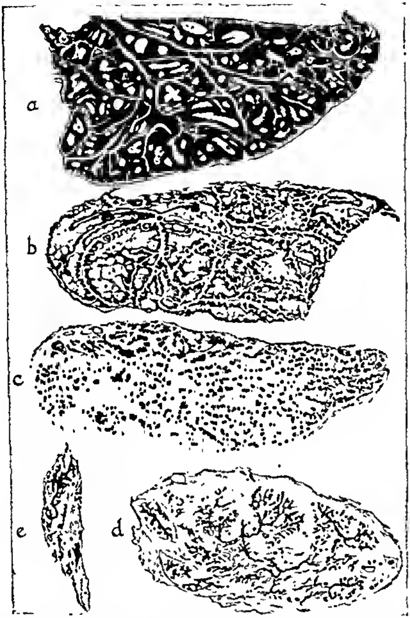

Fig. 272. Human thymuses at various ages showing age and accidental involution: *a*, From a newborn, weight of gland 15 gm ; *b*, from a seven-year-old boy, weight of gland 35 gm ; *c*, from a seventeen-year-old boy, weight of gland 35.2 gm, showing beginning age involution; *d*, from a seventeen-year-old boy, weight of gland 8.8 gm., high grade accidental involution; the dark parenchyma is surrounded by fat and connective tissue; *e*, from a seventeen-year-old boy, weight of gland 1.65 gm, very extensive accidental involution. Redrawn and slightly modified after Hammar (1906).

The thymus consists of two main lobes, one on each side of the median line, which are closely joined by connective tissue, but are not actually fused. Each of these lobes is divided into a number of macroscopic lobules varying from 0.5 to 2 mm. in diameter. The lobules are separated from one another by the interlobular connective tissue and are divided into a darkly staining, peripheral cortical area and an inner, lighter staining, medul-

cially the sex glands and the thyroid. The cortex hypertrophies during pregnancy. Sexual precocity in children and virilism in girls are frequently associated with overgrowth or hypertrophy of parts of the cortex. Animals with comparatively large testes have large adrenals. In rabbits the adrenal glands hypertrophy, sometimes to two or three times the normal size, after removal of the thyroid. The cortex of the adrenal hypertrophies after the action of those toxins which cause rapid atrophy of the thymus. The presence of both cortex and medulla seems necessary for thymic involution. The atrophic cortex which results from hypophysectomy regenerates after the injection of the adrenocorticotrophic hormone of the pars distalis. Removal of the adrenal interrupts lactation. In such animals salt in the diet and small amounts of cortical extract maintain lactation. Removal of the adrenal also stops the estrus cycle in rats. Cortical extract causes a return of the cycle. In Addison's disease there is a marked decrease in the number of basophils of the pars distalis. Adrenotrophin causes a retardation of chondro- and osteogenesis in the epiphyses of long bones of rats and regression of pituitary basophils. The cortex, especially the zona fasciculata, contains large amounts of ascorbic acid (vitamin C).

THE PARAGANGLIA (CHROMAFFIN SYSTEM)

Under this term are grouped several widely scattered accumulations of cells which seem to have much in common with the medullary cells of the adrenal glands. These paraganglia include widespread, small accumulations of cells in the retroperitoneum—the organs of Zuckerkandl, and collections of similar cells in the kidney, ovary, liver, testis, and heart. Most, but not all, authors believe they arise from sympathogonia and all contain chromaffin cells. The chromaffin cells are clear in the fresh condition or after most fixatives, but stain positively with chromic and osmic acids and contain iron. These cells are usually arranged in more or less definite cords and have a rich blood supply.

It has not been proved that these paraganglia have an endocrine function. The assumption that they elaborate epinephrine, just like the chromaffin cells of the medulla of the adrenal, has not been established. Some authors include the medullary cells of the adrenal in this group and speak of them all as the chromaffin system. The advisability of this must be questioned until it has been shown that all of the chromaffin organs have the same internal secretion.

The Carotid, Aortic and Coccygeal Bodies. These structures are often erroneously included with the endocrine glands. The carotid and aortic bodies are described on page 252 and the coccygeal body on page 246.

THYMUS

In man the thymus is an unpaired gland situated in the anterior mediastinum, in close connection with the pericardium and the great veins at the base of the heart. The thymus presents extreme variations in its structure which depend on the age and condition of the organism as a whole. Its function is not known and has been the subject of much speculation and experimentation. The organ is closely related to lymphatic tissue.

In relation to body weight, the thymus is largest during embryonic life and in childhood up to the period of puberty. After this it begins to involute—a process which proceeds gradually and continuously throughout life under normal conditions. This change in its structure is spoken of as *age involution*. During the course of infectious and cachectic diseases, the normal slow involution may be greatly accelerated. This is called *accidental involution* and explains many of the contradictory reports on the size of the thymus, since the organ did not have a

chance to regenerate in those individuals who died as a result of severe infections. At birth the thymus weighs 12 to 15 gm. This increases to about 30 to 40 gm. at puberty, after which it begins to decrease in weight so that at sixty years it weighs only 10 to 15 gm.

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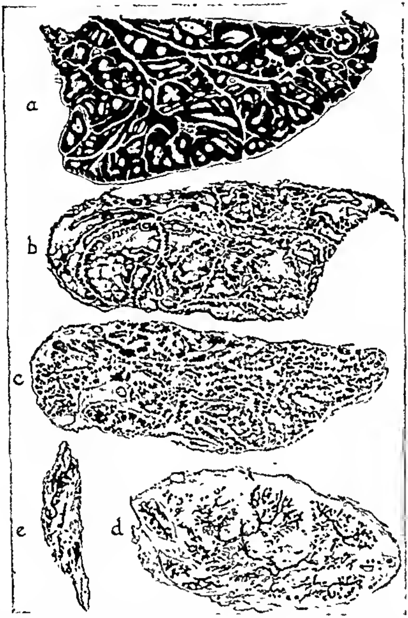

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lary portion (Fig. 272). With the study of serial sections one can trace a continuity of the medullary tissue from one lobule to another; that is, the medulla consists of a central stalk from which arise projections of medullary tissue; these are almost completely surrounded by a zone of cortical tissue.

Cortex. The cortical substance consists of densely packed masses of small cells, morphologically identical with the small lymphocytes; there are also cells identical with medium-sized and large lymphocytes. Some authors, however, deny the lymphocytic nature of these cells and call them *thymocytes*, for reasons that are discussed below. They have an average diameter of 6 μ, exceedingly small amounts of cytoplasm, a dark nucleus which consists of 5 to 6 heavy chromatin particles and a distinct nucleolus. Scattered between these small cells are elongated reticular cells with pale, round or oval nuclei. In most cases their nuclear membrane is smooth; the nucleus contains a few small chromatin particles and one or two nucleoli. It is difficult to follow the outlines of the cytoplasm of these cells, particularly when the surrounding lymphocytes are closely packed about them. As one proceeds from the cortex toward the medulla most of the lymphocytes disappear rather abruptly although there is no sharp line of demarcation between the two zones.

Medulla. The medulla consists predominantly of reticular cells similar to those of the cortex. As lymphocytes are much less numerous here, the outlines of the reticular cells can be traced more easily and they are seen to form a network with its meshes filled with lymphocytes. The medulla also contains the bodies of Hassall which are characteristic of the thymus (Fig. 273, *H*). They are rounded acidophil structures which vary from 30 to over 100 μ in diameter. They are composed of concentrically arranged cells, many of which show evidences of degeneration and hyalinization. Reticular cells are connected at one or more places with the periphery of each Hassall's body. The cells of the central part of a Hassall's body may degenerate completely so that small cysts may develop in the center. In other cases calcium may be deposited in them.

The medulla of the thymus is more vascular than the cortex, and surrounding each vessel are a few flat connective tissue cells. In addition to the lymphocytes, eosinophil myelocytes and plasma cells occur not infrequently. Very exceptionally, the thymus may contain lymphatic nodules (Jolly and Tannenberg).

Stroma. Most of the reticular cells of the thymus are of entodermal origin, although there are a few mesenchymal reticular cells about the blood vessels. Most of the reticular fibers are concentrated about the blood vessels and great masses of epithelial cells do not contain any of these fibers. Further study of the fiber content of the organ, particularly during involution, is necessary.

In the embryo the epithelial nature of many of the reticular cells is quite obvious (Fig. 275), but as the organ becomes more and more heavily infiltrated with lymphocytes, these epithelial cells become flattened and it is difficult to distinguish them from the nuclei of connective tissue reticular cells. A peculiarity of the thymic cellular reticulum is the fact that in vitally stained animals these cells in the thymus do not take up any of the dyestuff, while in certain of the diseases of malnutrition in infants, these reticular cells store large quantities of iron and fat, and in lipoid histiocytosis (Niemann-Pick disease) they become swollen with lipoid droplets. In experimental accidental involution the epithelial cells become loaded with dead lymphocytes.

The epithelial nature of the reticular cells becomes quite prominent when the lymphocytes have been destroyed by *x*-ray

and the epithelium begins to develop. It becomes even more prominent in transplants and tissue cultures of the thymus (Fig. 274). Certain tumors of clearly epithelial nature arise in this gland. In addition to the epithelial reticulum, there are macrophages and perivascular mesenchymal cells about the blood vessels of all sizes and in the interlobular septa.

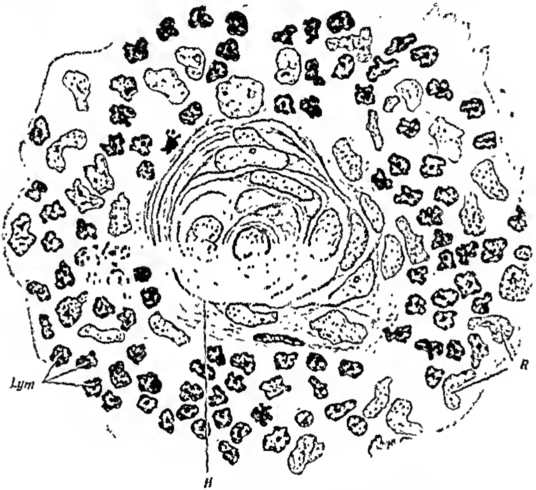

Fig. 273. Portion of the medulla of the thymus of an eight-year-old boy: *H*, Hassall's body; *R*, epithelial reticular cells; *Lym*, lymphocytes; an eosinophil myelocyte is just outside the left-hand margin of the Hassall's body. Eosin-azure stain. 970 ×. (W B.)

The Thymocytes. They are morphologically identical with lymphocytes. Both show the same susceptibility to *x*-ray injury; both are cytolyzed by sera obtained by the injection of thymus cells into rats; and both show the same type of ameboid motion and ability to transform into macrophages (Murray). Grégoire has found that transplants of the thymus consist only of epithelium if lymphocytes are prevented from migrating into them by mechanical means. Further, the transformation of the small thymocytes into plasma cells and eosinophil myelocytes is generally admitted. The mitochondria have the same appearance in both types of cells. However, in spite of these similarities, a few authors are not willing to classify the thymocytes as lymphocytes because they believe the thymocytes to have an epithelial origin, although most workers believe that they arise from lymphocytes which have wandered into the epithelium (see p. 322). Further work with pure cultures of thymic epithelium might clarify this moot question.

Involution of the Thymus. The above description of a clear-cut separation of the thymus into cortex and me-

dulla obtains normally in the later embryonic periods and in childhood. Normally involution begins as a gradual thinning out of the lymphoid cells of the cortex; at about four years the epithelial reticular cells become compressed, and individuals there are scattered Hassall's bodies surrounded by a few reticular cells and lymphocytes. This process of normal or age involution may be complicated by the rapid changes of "accidental involution" (Fig. 272).

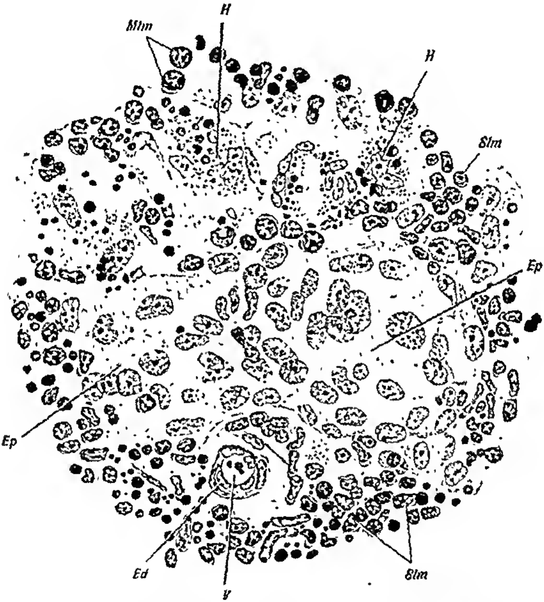

Fig. 274. Tissue culture of adult rabbit thymus, twenty-four hours *in vitro*, in a medium containing lithium carmine. The epithelial reticulum is contracting into a solid epithelial island (*Ep*); *Ed*, endothelium; *H*, macrophages with carmine granules and cellular débris; *Mlm*, medium-sized lymphocytes, *Slm*, small lymphocytes; *V*, vessel. 870 ×. After Popoff.

the area occupied by them is gradually replaced by adipose tissue, which is thought to arise in the interlobular connective tissue. The medulla begins to atrophy at puberty. This process continues throughout life. The last elements to be replaced are the Hassall's bodies, but even in very old

Purified adrenocorticotrophic hormone causes a striking reduction in weight and size of the thymus in male rats. Repeated injections of horse gonadotrophic hormone cause atrophy of the thymus through the intermediation of the interstitial cells of the gonads; this atrophy

results if the germ cells have been destroyed, but does not occur in castrated rats. On the contrary, castration causes hyperplasia of the involuted gland in the rat. Selye has found that fasting, toxins, and morphine cause a rapid atrophy of the thymus and enlargement of the adre-

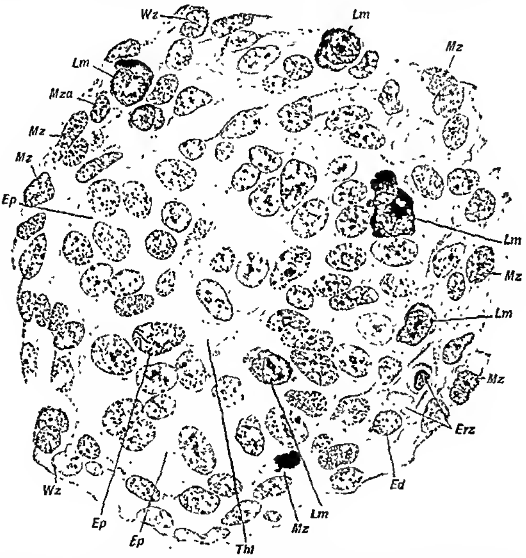

Fig. 275. Portion of a cross section through the right thymus of a 14.5 mm. rabbit embryo. The organ appears as an epithelial island (*Ep*) surrounded by mesenchyme (*Mz*); *Ed*, endothelium of a vessel containing a nucleated erythrocyte (*Erz*); lymphocytes (*Lm*) originate from the mesenchyme and wander into the epithelium; *Thl*, lumen of the thymus; *Wz*, histioid wandering cell. 900 ×. (A.A.M.)

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The regeneration of the thymus occurs rapidly after exposure to sublethal doses of *x*-rays and various toxins.

Vessels and Nerves. The arteries supplying the thymus arise from the internal mammary and the inferior thyroid arteries and are first distributed to the cortical tissue. Large venules arise in the medulla and combine into larger veins which empty into the left innominate and thyroid veins. The lymphatics run mainly in the interlobular connective tissue and empty into the anterior mediastinal and tracheobronchial lymph nodes. The thymus receives a few branches from the vagus and sympathetic nerves; these are probably mainly of vasomotor nature.

Histogenesis. In man the primordium of the thymus is an outgrowth of the third branchial pouch on each side of the median line; the fourth branchial pouch often gives rise to some thymic

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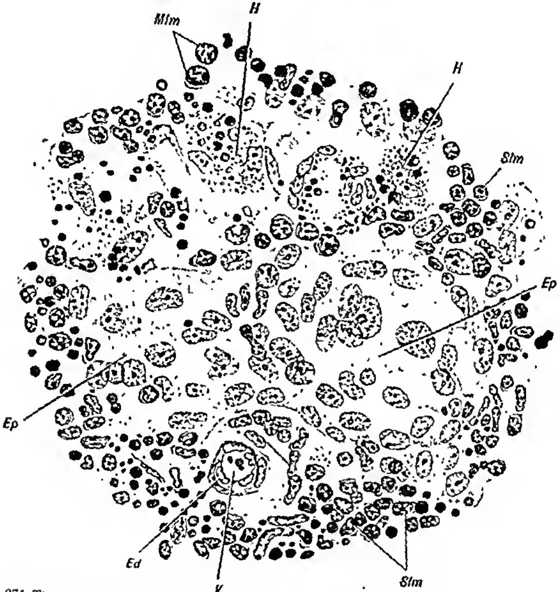

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Histogenesis. In man the primordium of the thymus is an outgrowth of the third branchial pouch on each side of the median line; the fourth brancial pouch often gives rise to some thymic

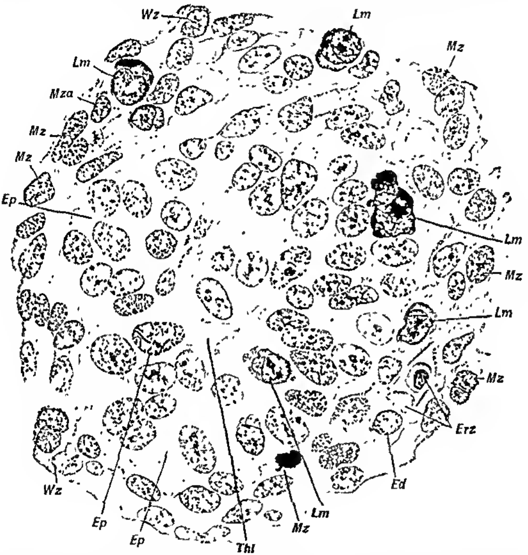

Fig. 275. Portion of a cross section through the right thymus of a 14.5 mm. rabbit embryo. The organ appears as an epithelial island (*Ep*) surrounded by mesenchyme (*Mz*); *Ed*, endothelium of a vessel containing a nucleated erythrocyte (*Erz*); lymphocytes (*Lm*) originate from the mesenchyme and wander into the epithelium; *Thl*, lumen of the thymus; *Wz*, histioid wandering cell. 900 ×. (A.A.M.)

tissue (Van Dyke, 1911). It has a narrow, cleft-like lumen (Fig. 275, *Thl*) and a thick wall which consists of several layers of cylindrical epithelium (Fig. 275, *Ep*). The surrounding mesenchyme, in the very earliest stages, contains many large lymphoid wandering cells (Fig. 275, *Lm*, *Wz*), which arise from the mesenchyme cells. The epithelial bud proliferates irregularly so that the lumen disappears and anastomosing strands extend into the mesenchyme. The future lobules arise at the ends of these branches.

In an embryo of about 20 mm., small thymocytes appear. A few authors derive them from the epithelial cells, but according to the theory which is dominant at present, the thymocytes arise from the inwandering of lymphoid cells. Some of them are quite large, while others are very small, and there are numerous transitions between the two (Fig. 275). It is almost certain, moreover, that many of the lymphoid cells arise from the perivascular mesenchymal cells of the blood vessels which penetrate the epithelium. The number of the lymphocytes increases greatly, partly from inwandering from the mesenchyme and in part through their own proliferation. With the continued growth of the organ, the lymphocytes become predominantly of the small lymphocyte type. The epithelial sheet is converted into a reticular cell mass whose meshes are occupied by the lymphocytes and are penetrated here and there by blood vessels.

The definitive medulla arises relatively late in the central main stem and in the deeper portions of the lobules by the hypertrophy of the syncytial mass of epithelium, while most of the lymphocytes move from these areas or degenerate *in situ*. Later, through as yet imperfectly described changes in the epithelial cells, the Hassall's bodies arise. The cortex and medulla arise from the same cell mass, but have a different distribution of lymphocytes.

In later stages of embryonic life, many lymphocytes wander into the blood and lymph streams and some of them turn into granulocytes. The thymus in the embryo, then, must be considered to be a blood-forming tissue even though no erythrocytes are produced in it. The embryonic thymus bears some points of similarity to the embryonic liver: in both organs the epithelial cells are separated by mesenchymal cells and lymphocytes. In both organs the lymphocytes produce granulocytes, although in the liver they also produce erythrocytes.

Most investigators ascribe the regeneration of the small thymocytes in transplants of the gland to immigration of lymphocytes (see Jolly, 1932).

Function of the Thymus. The functions of the thymus are unknown, except for its ability to form lymphocytes and a few plasma cells and myelocytes. But the change from a very large organ in the embryo, infancy, and childhood, into a gradually disappearing organ with the development of sexual maturity, has led many authors to ascribe an endocrine function to this gland. The claims that the injection of extracts of the thymus into parent rats causes a precocious growth of their progeny which is cumulative in succeeding generations and that thymectomy causes a retardation in growth of the progeny have not been substantiated.

THE PINEAL BODY

The pineal body (conarium, epiphysis cerebri) is a somewhat flattened, conical, gray body measuring 5 to 8 mm. in length and 3 to 5 mm. in its greatest width. It lies above the roof of the posterior extremity of the third ventricle, to which it is attached by the pineal stalk. The cavity of the third ventricle extends for a short distance into the stalk as the pineal recess; this is lined with ependyma.

Except where it is attached to the habenular and posterior commissures of the midbrain, the pineal body is invested by pia mater. Connective tissue septa, containing many blood vessels, arise from this layer, penetrate the pineal body and separate its specific elements into cords of cells. In hematoxylin-and-eosin-stained sections, the pineal body is seen to consist of cords of epithelioid cells with dark nuclei and little cytoplasm, embedded in a reticular framework. With advancing age, some of the small dark cells gradually develop into larger cells with much cytoplasm and paler nuclei.

In addition to the presence of neuroglial cells—on which all authors are agreed—five types of cells have been described: (1) chief cells, which are large and have small processes and no vacuoles in their homogeneous protoplasm; (2) smaller cells with fine acidophil granules; (3) cells with large basophil granules; (4) cells with

lipoid granules, and (5) nerve cells. Some authors claim that the various granules are evidences of a secretory process.

According to the recent studies of del Rio-Hortega on the pineal body, the parenchymatous cells of the organ are specific cells with a characteristic structure (Fig. 278). They have long processes which extend for considerable distances and end in bulblike swellings in the interlobular connective tissue (Fig. 278). In body. These parenchymatous cells arise from the same source as the neuroglia and nerve cells, but seem to be an intermediate cellular form.

It is generally believed that the pineal body increases in size until about seven years of age. At this time involution is said to begin and to continue to fourteen years of age. It is manifested by an increase in the amount of neuroglia and by the development of hyaline changes in

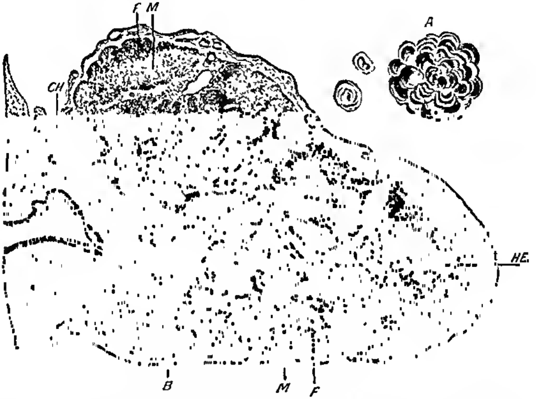

Fig 276 Median section through the pineal body of a newborn child; *B*, Connective tissue sheath (pia mater); *CH*, superior habenular commissure; *E*, ependyma; *F*, group of cells with little protoplasm; *G*, neuroglia; *HE*, posterior end of the pineal body; *M*, cells with much protoplasm; *RP*, pineal recess; *S*, connection with posterior commissure. Blood vessels empty. 32 ×. *A*, Acervulus from the pineal body of a woman of sixty-nine years. 160 ×. After Schaffer.

the center of the lobules, these processes radiate in all directions from the cells, while toward the periphery of the lobules the cells tend to become polarized in one or two processes. Occasionally, there are as many club-shaped ends of fibers in the center of the lobules as in the periphery; these may be considered evidences of atrophy. The specific elements hypertrophy greatly about the *sand granules* (Fig. 276, *A*) which develop in the pineal both the septa and the lobules. The so-called "brain-sand granules" (corpora arenacea) also begin to appear. These are laminated structures consisting mainly of phosphates and carbonates of calcium and magnesium.

The pineal body first appears at the beginning of the second month of gestation as a fold arising from the roof of the diencephalon before which is a collection of rounded cells. By the end of the sixth

month, these have differentiated into neuroglia cells and pineal cells.

The function of the pineal body is unknown. Some authors regard the pineal body as a vestigial organ homologous to the pineal sense organ of the lower vertebrates. This is denied by others who believe that the body is a gland of internal secretion and that its absence causes a marked increase in the rapidity of sexual development. Unfortunately, extirpation and injection of extracts of the organ have given inconstant and highly contradictory results. Typical secretory granules are not found in the specific pineal cells.

Fig 277. Section of pineal body of man stained with hematoxylin and eosin, showing irregularly shaped cells and their processes. Note blood vessel in the center Compare with Fig. 278.

Fig. 278. Specifically impregnated section of pineal body of a young boy showing, C, interlobular tissue, and, D, vessel with club-shaped processes of specific cells in its adventitia. Note parenchymatous cells and their claviform processes bordering on C. After del Rio-Hortega.

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Chapter XV

THE SKIN

THE skin covers the surface of the body and consists of two main layers, the surface epithelium or the *epidermis* and the subjacent, dense connective tissue layer—the skin proper, *corium* or *derma*. Beneath the latter is a looser connective tissue layer, the superficial fascia or *tela subcutanea*, which in many places of the body is transformed into the subcutaneous fatty tissue, the *panniculus adiposus*. The superficial fascia may be called the *hypodermis*, which is more or less closely connected with the deeper tissues such as the deep fasciae, aponeuroses, or periosteum.

The skin is continuous with several mucous membranes through mucocutaneous junctions, the most important of which are the vermilion border of the lip, the vulva and the anus.

The skin carries out several functions: it protects the organism from injurious external influences; it receives sensory impulses from the outside; it excretes various substances to the outside, and in warm-blooded animals, it helps to regulate the temperature of the body. The skin is provided with numerous accessory organs, such as the hairs, nails, and glands of various kinds.

On the free surface of the skin, in man, numerous ridges can be seen with the naked eye, which pass in various directions, cross one another in the form of a network, and frequently unite. On the soles, palms, and undersurfaces of the fingers and toes, in man and the primates, there is a regular pattern of parallel ridges which form complicated figures. This pattern in its fundamental plan is always the same, but undergoes marked individual variations, so that it is different in every person.

The boundary between the epithelial and the connective tissue portions of the skin is well pronounced. But it is impossible to draw a sharp histologic boundary between the derma and the subcutaneous layer; the fibers of one layer pass directly over into the other layer.

The surface of contact between the epidermis and the derma is uneven in most places. The boundary between the two layers appears as a straight line only on the forehead, the midline of the perineum and scrotum, and the external ear. In most of the skin of the body the outer portion of the derma is provided with a series of irregular ridges called *papillae;* into the spaces between them the lower layers of the epidermis intrude.

EPIDERMIS

The epidermis is a stratified squamous epithelium, the external layer of which hornifies and forms a resistant surface. It is moistened by water only with difficulty and prevents the underlying tissues from drying; it thus serves as a protective layer.

The epidermis varies from 0.07 to 0.12 mm. in thickness on most parts of the body, although on the palms and the palmar surface of the fingers it may reach a thickness of 0 8 mm. and on the sole and toes of 1.4 mm. Continuous rubbing

and pressure through heavy physical work cause a great thickening of the epidermis, external mechanical causes, for it is well developed in the fetus.

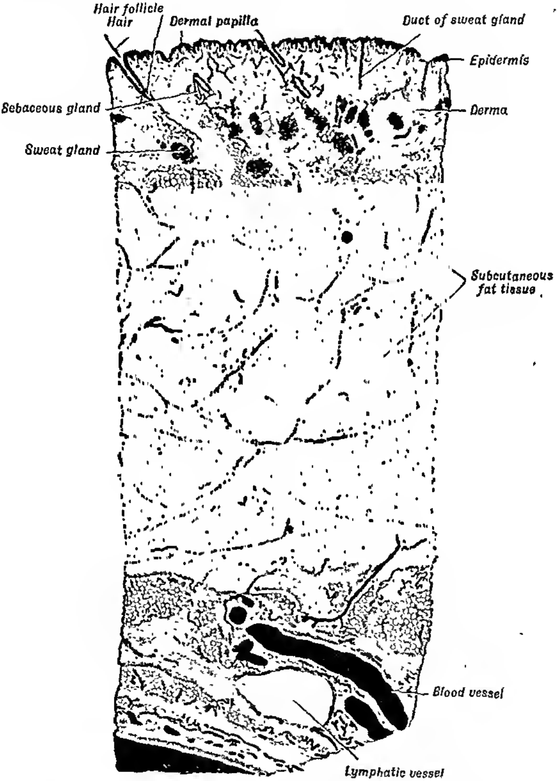

Fig. 279. Section through a human thigh perpendicular to the surface of the skin. Blood vessels are injected and appear black. Low magnification. (A.A.M.)

especially of its horny layer. The great thickness of the epidermis on the palms and the soles cannot be the result of only

Epidermis of the Palms and Soles. The structure of the epidermis is most typical in those places where it is thickest.

Here, in sections perpendicular to the surface, four main layers can be distinguished: (1) the deep layer of Malpighi (stratum germinativum or spinosum) touching the derma; (2) the granular layer (stratum granulosum); (3) the clear layer (stratum lucidum); and (4) the horny layer (stratum corneum).

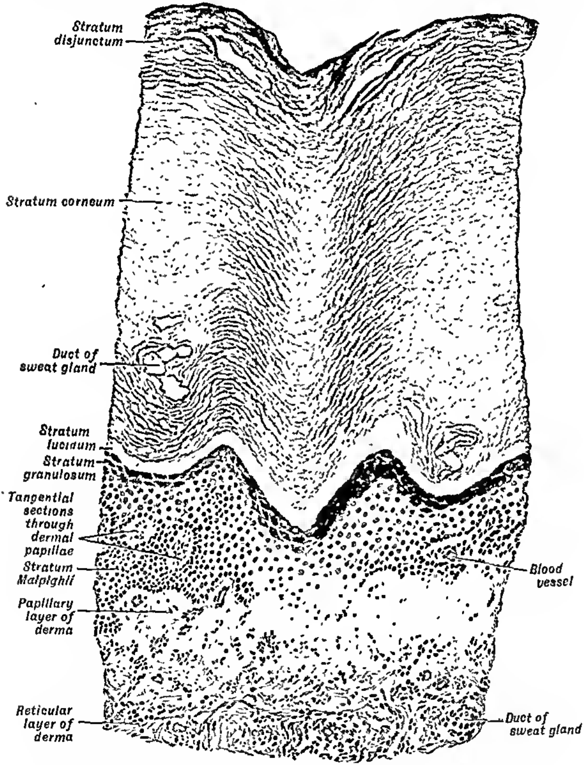

Fig. 280. Section of a human sole; perpendicular to the free surface. 100 ×. (A.A.M.)

The *Malpighian layer* (Fig. 280) is thicker between the dermal papillae than above them. The deepest or basal layer of cells, adjacent to the derma, consists of cylindrical cells placed perpendicularly to the surface of the skin; mitotic figures occur rather frequently. Higher up the cells become polyhedral and they are flat-

tened under the granular layer. The surface of these cells is covered with thin spines (hence the name, *stratum spinosum*) which connect with similar spines of adjacent cells and form bridges crossing the intercellular spaces (Fig. 281, *y*). The spines at the lower surface of the cylindrical layer of cells are finger-like processes which project into the connective be thickened into a round or spindle-shaped granule.

The granular layer (Fig. 280) consists of three to five layers of flattened cells which are rhomboidal in a perpendicular section. Their cytoplasm, particularly in the vicinity of the nucleus, contains irregularly shaped granules of keratohyalin. Their origin has not been definitely estab-

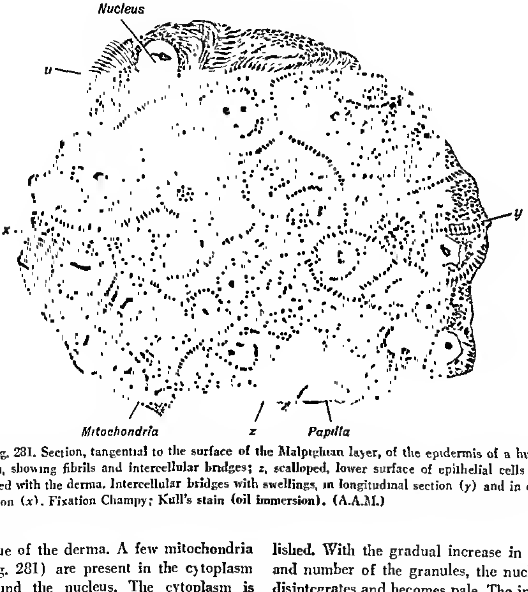

Fig. 281. Section, tangential to the surface of the Malpighian layer, of the epidermis of a human palm, showing fibrils and intercellular bridges; *z*, scalloped, lower surface of epithelial cells connected with the derma. Intercellular bridges with swellings, in longitudinal section (*y*) and in cross section (*x*). Fixation Champy; Kull's stain (oil immersion). (A.A.M.)

tissue of the derma. A few mitochondria (Fig. 281) are present in the cytoplasm around the nucleus. The cytoplasm is also provided with numerous fibrils, which are arranged in parallel bundles, particularly in the peripheral layer of the cell body. The fibrils pass through the intercellular bridges and penetrate rows of cells without interruption. Within each intercellular bridge, the fibril appears to lished. With the gradual increase in size and number of the granules, the nucleus disintegrates and becomes pale. The intercellular spaces at the same time become narrow and the intercellular bridges, maintaining the swellings in their middle, become shorter and rather indistinct.

The *stratum lucidum* (Fig. 280) consists of several layers of flattened, closely packed cells; in a section it appears as a

pale, wavy stripe, in which the granules of keratohyalin have dissolved and become eleidin. In the majority of cases nothing remains of the nucleus in such cells.

The horny layer, the *stratum corneum* (Fig. 280), on the palms and soles reaches a considerable thickness and consists of dead, cornified, flattened cells. The intercellular bridges are absent here and the close contact depends on the dense arrangement of the cells which touch one another with their margins covered with small, irregular spines. The mass which fills the cells of the horny layer is paraeleidin or keratin, a product of the transformation of eleidin.

The most peripheral layers of the stratum corneum consist of dried. horny plates which are constantly being desquamated (*stratum disjunctum*). The cells lost in this way are replaced by new ones which come up from the lower layers. The number of mitotic figures in the deep layers of Malpighian cells corresponds with the intensity of the desquamation of the epidermis at the particular region of the skin.

Against the general view that mitosis in the skin occurs mainly in the basal layer of cells, Thuringer found that in the skin of the scalp and prepuce only 12 per cent of the mitoses were in the basal layer, with 30 per cent in the lower third, 46 per cent in the middle third and 12 per cent in the outer third of the spinous layer. In the palmar and plantar skin of the cat he found the mitoses slightly more numerous in the lower third of the spinous layer than in the basal layer. Both layers respond rapidly with an increased number of mitoses to mechanical stimulation of the skin in these areas.

Epidermis of the Body. On the rest of the body the epidermis remains much thinner and has a much simpler structure. Two layers are always present—the stratum Malpighii and the stratum corneum. The granular layer in the majority of cases consists of but one layer of cells and is frequently absent (Fig. 282). This depends on the fact that the process of cornification, *i. e.*, the transformation of cells of the Malpighian layer into cells of the corneum, does not proceed continuously, but occurs from time to time at different places. In contrast to what happens in the palms and soles, the epidermal cells in the other portions of the surface of the body produce very little eleidin so that they become thin plates of keratin with only a thin slit in the place of the former nucleus.

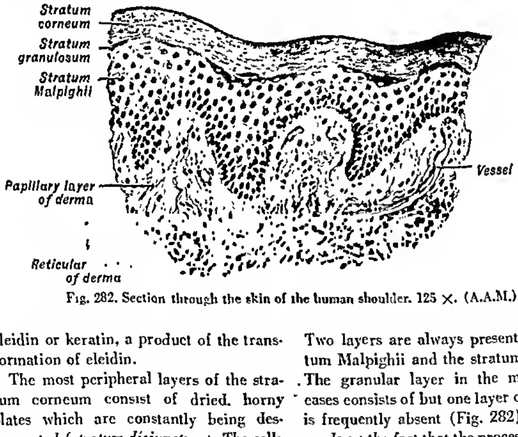

Fig. 282. Section through the skin of the human shoulder. 125 ×. (A.A.M.)

These very thin plates are welded so closely together that they are very hard to isolate, even after prolonged maceration.

The epidermis is entirely devoid of blood vessels and is nourished by the tissue fluid which penetrates into the intercellular spaces of the Malpighian layer from the capillaries in the underlying connective tissue.

The color of the skin depends on three factors: Its inherent color is predominantly yellow; the vascular bed gives a reddish hue; *melanin* is responsible for varying shades of brown. This pigment accumulates in smaller or larger amounts as fine granules within the cells of the stratum germinativum, particularly in its basal, cylindrical layer (Fig. 283, *Pb*). As these cells move toward the surface, this granular pigment gradually disappears, so that only a diffuse coloring is observed in the stratum corneum. The pigmentation of the skin of the Negro is due to the great amount of pigment in all of the layers of the epidermis.

At the junction of the layer of pigmented basal epithelial cells with the derma, and projecting slightly into the latter, are peculiar branched pigment cells called "melanoblasts" (Fig 283, *Mel*). The long axis of their nucleus is usually perpendicular to that of the basal cells and their pigment-containing processes extend for surprising distances between the epidermal cells. These *melanoblasts* react positively and specifically with the "dopa" reagent—3, 4 dioxyphenylalanin—while the pigment cells of the Malpighian layer and the *dermal chromatophores* (see below) do not Exposure to x-rays and ultraviolet light increases the "dopa" reaction in the melanoblasts It has been suggested that the melanoblasts are specific cells that normally elaborate melanin which they turn over to the epidermal cells According to one author the melanoblasts are merely altered basal cells.

Throughout the epidermis, but more frequently in its upper layers, peculiar black, star-shaped figures can be seen in gold chloride preparations. They are provided with long, irregular processes which penetrate the intercellular spaces and follow the intercellular outlines. They are the so-called *cells of Langerhans*. They have been considered, probably erroneously, as melanoblasts, nerve cells, or lymphoid wandering cells by different investigators. It is not at all clear, however, that they are cells No structural details

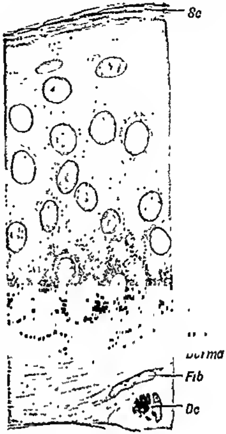

Fig. 283. Section through the skin of a human mammary papilla: *Sc*, Stratum corneum; *Pb*, pigmented basal cells of epidermis; *Mel*, melanoblast; *Fib*, fibroblast; *Dc*, dermal chromatophore. Silver nitrate, faintly counter-stained with pyronin methyl green. 650 ×. (W. B.)

can be seen in them in gold-impregnated slides and no traces of them can be found in slides prepared by the usual histologic methods.

Basement Membrane. Most recent authors deny the presence of a basement membrane between epidermis and derma. The opinion that the tonofibrils of the epidermis are directly connected with the collagenous fibers of the derma has not been proved.

Mucocutaneous Junctions. These junctions differ in several respects from the skin and the mucous membranes to which they are joined. Their epithelium is thicker than that of the adjacent skin and more nearly resembles that of the mucosa. Normally, they contain no sweat or mucous glands, but do have superficially placed sebaceous glands in perhaps half

of the cases. They are moistened by mucous glands within the orifices. As the lip has a thin stratum corneum and normally lacks a stratum granulosum, the underlying blood shines through and gives it a red color.

THE DERMA

The thickness of the derma cannot be measured exactly because it passes over directly into the subcutaneous layer. The average thickness is approximately 1 to 2 mm.; it is less on the eyelids and the prepuce (up to 0.6 mm.), but reaches a thickness of 3 mm. or more on the soles and palms. On the ventral surface of the body and on the underside of the appendages it is generally thinner than on the dorsal and the upper sides; it is thinner in women than in men.

The surface of the derma which is fused with the epidermis is uneven in most cases and is covered with projecting ridges and papillae. This surface layer of the derma is soft and is called the *papillary layer* (Fig. 280). The deep layer, *i. e.*, the main dense portion of the derma, is called the *reticular layer* (Fig. 280). The two layers cannot be clearly divided from each other.

The reticular layer consists of bundles of collagenous fibers which form a dense feltwork; the bundles run in various directions (Fig. 57), of which the main one is always more or less parallel to the surface; less frequently, approximately perpendicular bundles are found. In the papillary layer and its papillae the collagenous bundles are much thinner and more loosely arranged.

The elastic fibers of the derma form abundant, thick networks in the space between the collagenous bundles and are condensed about the hair follicles and the sweat and sebaceous glands. In the papillary layer they are much thinner and form a continuous fine network under the epithelium in the papillae. In the cheeks, however, the elastic network immediately under the epithelium is particularly dense and consists of closely arranged, markedly twisted fibers.

The cells of the derma are the same as those of the subcutaneous layer (p. 59); in the papillary layer they are more abundant than in the reticular layer.

In the peripheral layers of the derma, under the epithelium, a few connective tissue pigment cells, *dermal chromatophores*, are scattered (Fig. 283, *Dc*). In these cells the pigment granules are much larger and more irregular than the small, even granules in the melanoblasts and epidermal cells. The dermal chromatophores are encountered normally but rarely, and are numerous only in definite places, as around the anus. Whether these pigmented cells of the derma have anything to do with the pigment in the epidermis is not established. It is unlikely that they supply the pigment to the epithelial cells of the Malpighian layer; on the other hand, it is possible that the chromatophores receive their pigment from the epithelium. These chromatophores probably do not elaborate the pigment which they contain. In the skin of the ape, in the so-called "Mongolian spots" and in certain tumors, called "blue nevi," true dermal melanoblasts appear. These cells give a positive "dopa" reaction, in contrast to the "dopa" negative dermal chromatophores.

Within the deep parts of the reticular layer in the mammary papillae, the penis, perineum, and scrotum, numerous smooth muscle fibers are collected into a netlike layer. Such portions of the skin become wrinkled during contraction of these muscles. In the peripheral layers of the derma, smooth muscles are also connected with the hairs (p. 339).

In many places in the skin of the face, cross-striated muscle fibers terminate in the derma.

At various levels of the derma are the hair follicles, sweat and sebaceous glands, as well as blood vessels and nerves and many nerve endings. These different structures are described later in this chapter.

Hypodermis. The subcutaneous layer consists of loose connective tissue and is a loose continuation of the derma. The collagenous fibers and a few elastic fibers pass directly over into the fibers of the derma and run in all directions, mainly in that parallel to the surface of the skin. Where the skin is very flexible the fibers

are few; where it is closely attached to the underlying parts, as on the soles and the palms, they are thick and numerous.

Depending on the portion of the body and the nutrition of the organism, a varying number of fat cells develop in the subcutaneous layer (Fig. 279). These are also found in groups in the deep layers of the derma The fatty tissue of the subcutaneous layer on the abdomen may reach a thickness of 3 cm. or more, while in the eyelids and penis the subcutaneous layer never contains fat cells.

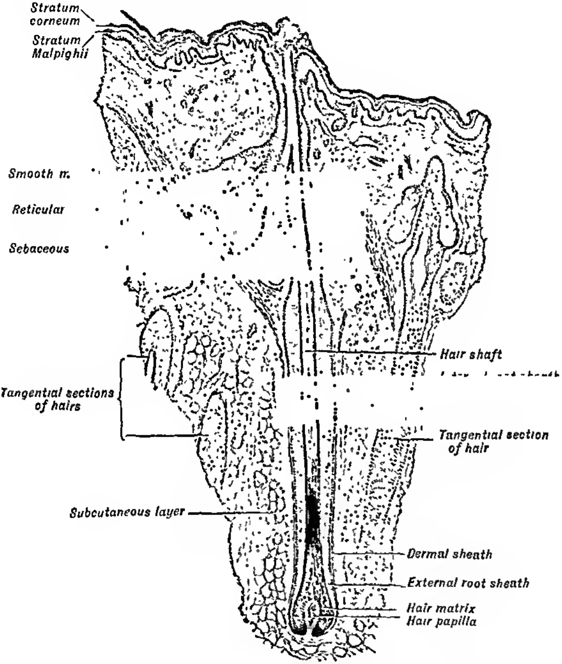

Fig 281. Scalp of a man Root of a hair in longitudinal section. 32 ×. After Schaffer.

The subcutaneous layer is penetrated everywhere by large blood vessels and nerve trunks and contains many nerve endings.

HAIRS

The hairs are horny threads which develop from the epidermis. They vary in length from several millimeters to 1.5

meters and from 0.005 mm. up to 0.6 mm. in thickness. They are distributed in varying density *on the surface of the skin,* except on the palms and soles, the lower and lateral surfaces of the fingers and toes, the upper surface of the third phalanx of all the fingers, the lips, the glans penis, the prepuce, and the internal surface of the labia majora.

Each hair arises in a tubular invagination of the skin, the *hair follicle*, the walls of which are composed of epidermis and derma. The connective tissue papilla projects into the bottom of the follicle. The are oval and sometimes irregularly angular in cross section. The form of the hair *does not correspond with its shape in* cross section. The shape of the hair is not a reliable indication of the race of the individual. The natural, free end of the shaft of each hair gradually thins down into a point.

The epidermis, which extends inside the hair follicle as far as the opening of the sebaceous gland, *i. e.*, the neck of the follicle, directly adjoins the shaft of the hair and consists of the usual Malpighian, granular, and corneum layers. Below the

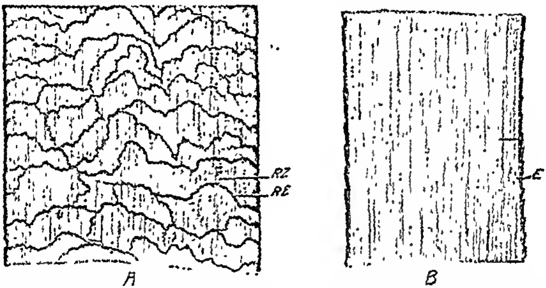

Fig. 285. Shaft of a human hair seen from the surface (A), and in profile (B), after treatment with 5 per cent NaOH: RZ, Cortical cuticular scales from the surface and in cross section (E); RE, outlines of the cuticles. 380 ×. After Schaffer.

root of the hair develops into the hair *shaft*, the free end of which protrudes beyond the surface of the skin (Fig. 281).

One or more sebaceous glands are connected with each hair. They are usually located in the obtuse angle between the hair follicle and the surface of the skin and open into the *neck* of the follicle. Here one end of a smooth muscle is attached to the middle of the connective tissue sheath of the follicle while the other end disappears in the papillary layer of the derma.

The external form of the shaft of the hair is usually cylindrical and appears as a circle in transverse sections. Many hairs neck, the corneum layer disappears and so does the granular layer shortly afterward. As a result, in the deeper portion of the hair follicle, only the Malpighian layer remains. Toward the base of the follicle it forms the *external root sheath* of the hair. This is separated from the root of the hair by the *internal root sheath* which grows upward with the hair from the base of the follicle to its neck, where it terminates with a free edge. The root and the internal and external sheaths blend at the surface of the papilla into a mass of undifferentiated epithelial cells, the *matrix* of the hair.

Structure of the Hair. The hair is covered with a special membrane, the

cuticle, which consists of thin, transparent, cornified epithelial cells with wavy outlines. These are arranged like shingles with their free margins directed toward the end of the hair.

The main mass of the hair consists of dense, horny substance, which contains a variable amount of pigment and air vacuoles. Concentrated sulfuric acid disintegrates it into flattened, spindle-shaped

Fig. 286. Longitudinal section through a hair from the head of a man of twenty-two years: *1*, Medulla; *2*, cortex; *3*, hair cuticle; *4*, inner sheath cuticle; *5*, Huxley's layer; *6*, Henle's layer; *7*, external root sheath, *8*, glassy membrane; *9*, connective tissue of the hair follicle; *AW*, external root sheath at the bulb, *A*, matrix; *P*, papilla. 350 ×. After Hoepke.

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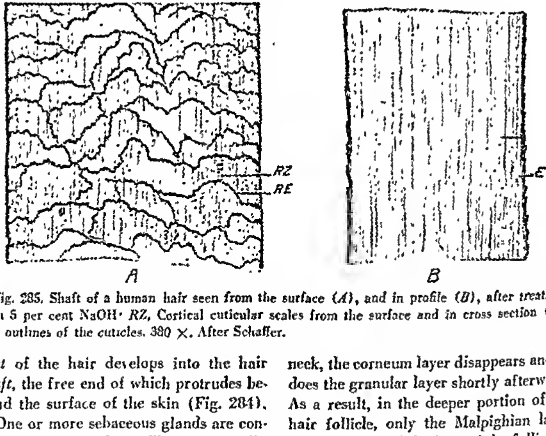

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1 2 3 4 5 6 7 8 9

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bodies containing dense bundles of fine, horny threads—the remains of intercellular bridges. Vestiges of the nucleus appear as shrunken rods or granules. The pigment is distributed diffusely or in rows of granules in the spindles. The air vacuoles are arranged in rows in the former intercellular spaces.

The horny tissue is usually called the *cortical substance* of the hair. This name, however, is sometimes inappropriate, for in the hairs on the head this "cortical" substance and the cuticle are the sole constituents of the hair shaft.

In many of the thick hairs (in beards and eyebrows) the axial portion is occupied by the *medulla*, composed of irregular, shrunken, cornified cells separated by large amounts of air and connected by bridges.

The color of the hair depends on its content of pigment and air. The larger the amount of pigment, the darker is the hair. Light hairs appear still lighter due to the presence of a medulla filled with air. The loss of pigment makes the hair look gray. If this happens in hairs which are rich in medullary substance the hair acquires a bright, silvery appearance.

The Hair Follicle. The connective tissue portion of the wall of the hair follicle is formed by a condensation of the derma bounding the invaginated epithelium, and is made up of three layers. The external, poorly outlined layer consists mainly of longitudinal collagenous and elastic fibers and a few fibroblasts. The thick, dense middle layer is formed by circular fibers and fibroblasts. The internal layer is a basement membrane between the epithelial and connective tissues (glassy membrane).

The *papilla* at the bottom of the follicle is an outgrowth of the connective tissue sheath of the latter and is homologous to an ordinary dermal papilla. It consists of a cellular connective tissue which contains capillaries and is usually devoid of elastic fibers. Sometimes it contains pigment cells, particularly in dark-haired people and Negroes. The glassy membrane becomes much thinner on its surface. The papilla is usually egg shaped, with a thin pedicle.

The surface of the papilla is covered with a continuous epithelial mass, the *hair matrix*, from which the hair and its sheaths develop. The cells of the deeper layer have a cylindrical form, but usually appear as a syncytial mass. The nuclei are often in mitosis. The cytoplasm of the cells at the tip of the papilla contains a number of pigment granules.

The cells which cover the papilla move upward and form the main mass of the hair. They become long, thin, pigmented spindles and bundles of fibrils appear within them and pass from cell to cell. At the boundary between the lower and middle thirds of the root, they are transformed into the cornified elements of the hair. Cornification proceeds here without the participation of a granular substance like keratohyalin or trichohyalin.

In hairs with a medulla, the cells at the tip of the papilla move forward as a column of polyhedral cells interconnected by bridges. Then, granules of trichohyalin appear in them. Higher up, these granules disappear and the cells become cornified, shrunken bodies between which air accumulates.

The cuticle of the hair develops from cells slightly below the middle of the side of the papilla. They lack pigment and at first are flat, but later become cubical; they gradually become wider and overlap one another with their free margins to the outside. The nuclei disappear and the cells become homogeneous, horny scales, closely welded to the cortical substance of the hair.

Internal Root Sheath. The next layers of cells down the slope of the papilla move upward and form the three layers of the internal root sheath. This, too, grows upward, but disintegrates below the opening of the sebaceous gland into the follicle.

The innermost layer, the *cuticle of the internal root sheath*, like the cuticle of the hair, consists of thin, horny scales, which overlap one another so that their free margins are directed toward

the bottom of the hair sac. They thus overlap the margins of the scales of the cuticle of the hair root which are directed toward the outside.

The cells which form the second layer or *layer of Huxley*, as they move from the lower part of the surface of the papilla, first become cuboidal and later spindle shaped. They are penetrated by thin fibrils and are connected with one another by bridges. At the level of the summit of the papilla they develop coarse trichohyalin granules. This substance remains for a distance of 40 to 50 cells in the layer of Huxley, but then

External Root Sheath. The external root sheath at the neck of the papilla consists of one layer of flat cells. Moving upward, it becomes two layered at the level of the middle portion of the papilla and further on stratified so that the external cells adjacent to the basement membrane become cylindrical. It was formerly believed that this layer of the epithelium grows from the glassy membrane toward the root, that

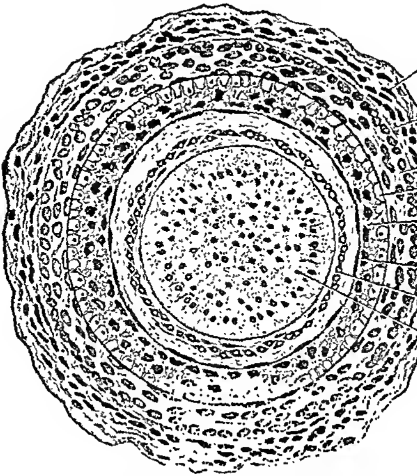

Fig. 287. Cross section through a hair follicle, in the skin of a pig embryo, at the level where Henle's layer is completely cornified and Huxley's layer contains granules of trichohyalin. 375 ×.

disappears as the cells cornify. At the middle third of the follicle, the layer of Huxley consists of one to three layers of irregular, cornified cells.

The outermost layer (*of Henle*) consists of a single layer of elongated, horny bodies connected by intercellular bridges. Their flat outer surface is closely welded to the external sheath. Sometimes, free spaces remain as oval openings between the edges of these cells. In this layer trichohyalin appears at about the middle of the papilla, but disappears at the level of the summit of the papilla.

is, perpendicularly to the direction of growth of the internal root sheath. Since intercellular bridges were discovered between the two sheaths, it is possible that the elements of the external sheath gradually move toward the exterior from the bottom of the hair sac.

The Muscle of the Hair. The hair muscle is called the *arrector pili* and consists of smooth muscle cells gathered into a cylindrical or band-

like shaft. It connects with its place of attachment by networks of elastic fibers. When this muscle contracts (from cold, etc.) it first transfers the shaft of the hair from its sloped position into a more vertical one; it then produces, in the region of its attachment to the surface of the derma, a groovelike invagination of the surface of the skin while the region surrounding the hair is lifted. This is responsible for the so-called "goose flesh." Finally, by pressing on the base of the sebaceous gland, it effects the liberation of a fatty material, which lubricates the shaft of the hair.

Replacement of Hairs. Throughout life, even in the embryo, hairs undergo replacement. The hair of every part of the body has a definite period of growth—the hairs of the head two to four years, the eyelashes three to five months. In man this change goes on continuously and passes unnoticed; in those mammals which replace their hair twice a year the process of replacement is very rapid.

When the period of growth of the hair approaches its end, the multiplication of the undifferentiated cells at the bottom of the hair sac in the matrix slows down and finally stops. The base of the shaft and the root gradually become thinner. The growth from below of the layers of the internal sheath stops. The elements which cover the summit of the papilla are all transformed into cornified spindles and the hair *becomes club shaped*. The *root gradually separates* from the papilla and moves slowly upward toward the neck of the follicle. The papilla becomes smaller and, according to some, atrophies and disappears. After having moved upward to the neck of the sac, the hair either falls or is pulled out.

Usually, even before the dropping out of the old hair, the primordium of the *new one* is formed in the same hair sac. The epithelium of the matrix begins to multiply, the lower portion of the follicle again becomes thicker and longer. The papilla enlarges, or as some believe, a new one is formed, and invaginates the epithelium at the bottom of the follicle. The cavity of the follicle above the papilla is soon filled with a mass of young epithelial elements. Inside this mass a layer of hornified cells, filled with trichohyalin, becomes visible; this has the shape of a hollow cone opened in the direction of the papilla. This layer represents the internal root sheath of the future new hair. The cells beneath this then form the substance of the hair proper.

Rats fed a diet deficient in zinc are reported to show hyperkeratinization of the skin with loss of hair follicles, although the sebaceous glands persist.

NAILS

The nails are approximately rectangular, horny plates on the dorsal surface of the terminal phalanges of the fingers and toes. The surface of the skin which is covered by them is called the *nail bed*. It is surrounded laterally and proximally by a fold of skin, the *nail wall*. The slit between the wall and the bed is called the *nail groove*. The proximal edge of the nail plate is the *root* of the nail. The visible part of the nail plate is surrounded by the nail wall and is called the *body of the nail*; the distal portion of it comes forward freely and is either gradually worn off by friction or is cut off. The nail body is pink because it is partially transparent and shows the underlying tissue rich in blood vessels. Near the root the nail has a whitish color; this portion, the *lunula*, is usually covered by the proximal portion of the nail fold; it frequently appears only on the thumb.

The dense, semitransparent nail plate consists of closely welded, horny *scales*; these are cornified epithelial cells. When treated with sodium hydroxide they separate and swell; it is then possible to distinguish in them the remains of a shrunken nucleus. These scales are arranged in layers so that in perpendicular sections the substance of the nail appears striated. Between the scales, air often accumulates in vacuoles and causes the appearance on the nail of irregular white spots.

The *nail fold* has the structure of skin with all its layers. Turning inward into the nail groove, it loses its papillae and the epidermis loses its horny, clear, and granular layers. Under the proximal fold, the horny layer touches the dorsal surface of the nail root and even spreads onto the free surface of the nail body as the eponychium (Fig. 288). The stratum lucidum and the stratum granulosum also reach

far inside the groove, but do not continue along the lower surface of the nail plate. On the surface of the nail bed only the Malpighian layer of the epidermis is present.

In the nail bed the derma is directly fused with the periosteum of the phalanx. The surface of the derma under the proximal edge of the nail is provided with rather low papillae but upper layer of cells which touches the substance of the nail is separated from it in places by an even line while in others it is jagged Under the free edge of the nail the usual horny layer again begins; it is thickened at this place and is called *hyponychium* (Fig. 288).

The epithelium which lines the proximal portion of the nail bed and corresponds roughly with the lunula is par-

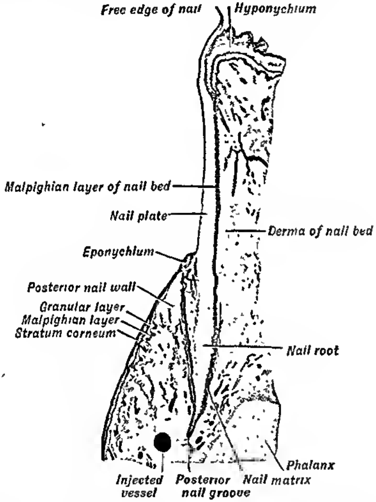

Fig. 288 Longitudinal section of a nail of a newborn infant. Very low power. (A.A.M.)

under the distal half of the lunula this surface is quite smooth. At the distal margin of the lunula, longitudinal, parallel ridges project instead of papillae. The boundary between the epithelium and the derma of the nail bed in a perpendicular section is, therefore, scalloped (Fig. 289) while it is smooth in longitudinal sections. Beyond the free edge of the nail the dermal ridges are replaced by cylindrical papillae.

The epithelium of the nail bed distal to the lunula retains the typical structure of the Malpighian layer. The *epithelium is thicker between* the ridges of the derma than over them. The

ticularly thick; distally and upward it gradually passes over into the substance of the nail plate. Here the new formation of the nail substance proceeds; accordingly this region of the epithelium is called the *nail matrix* (Fig. 288). The cells of the deepest layer are cylindrical and mitoses can be observed frequently in them. Higher up are six to ten layers of polyhedral cells, and *five to twelve* layers of flatter cells join them; this entire mass

is penetrated by parallel fibrils which consist of a special "onychogenic" substance. On passing into the substance of the proximal edge of the nail plate, these cells lose their fibrillar structure, cornify, and become homogeneous.

As the new formation of the nail takes place in the matrix, the nail moves forward. Although most authors deny the participation of the epithelium of the other portions of the nail bed in the formation of the nail substance, and believe that the nail simply glides forward over this region, some claim that the nail may be thickened by the addition of separate cornified elements.

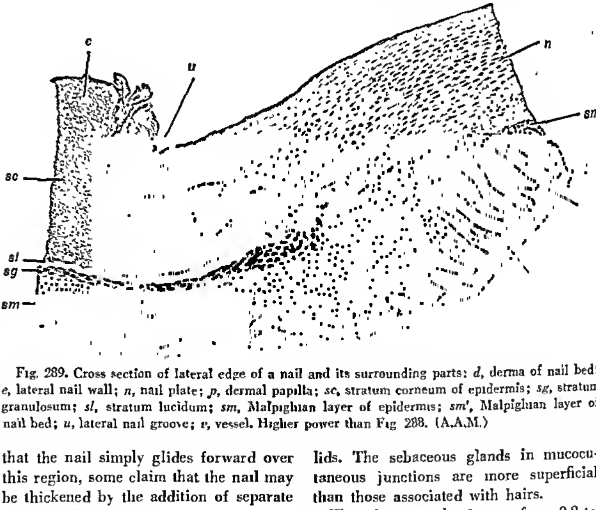

Fig. 289. Cross section of lateral edge of a nail and its surrounding parts: *d*, derma of nail bed; *e*, lateral nail wall; *n*, nail plate; *p*, dermal papilla; *sc*, stratum corneum of epidermis; *sg*, stratum granulosum; *sl*, stratum lucidum; *sm*, Malpighian layer of epidermis; *sm'*, Malpighian layer of nail bed; *u*, lateral nail groove; *v*, vessel. Higher power than Fig 288. (A.A.M.)

GLANDS OF THE SKIN

In man the glands of the skin include the sebaceous, sweat, and mammary glands. The latter are described in a separate chapter.

Sebaceous Glands. The sebaceous glands are scattered over the surface of the skin (except in the palms and soles). They lie in the derma and their excretory duct opens into the neck of a hair sac. When several glands are connected with one hair, they are closely placed and lie at the same level. On the lips, about the corners of the mouth, on the glans penis, and the internal fold of the prepuce, on the labia minora, and on the mammary papilla the sebaceous glands are independent of hairs and open directly on the surface of the skin; to this category also belong the Meibomian glands of the eyelids. The sebaceous glands in mucocutaneous junctions are more superficial than those associated with hairs.

The sebaceous glands vary from 0.2 to 2 mm. in diameter; the largest, such as those on the nose, are not accessories of the hairs, but, on the contrary, each is provided with a very small hair.

The secretory portions of the sebaceous glands are rounded sacs (alveoli). As a rule, several adjacent alveoli form a mass like a bunch of grapes, and all of them open into a short, excretory duct; in this

way a simple branched gland results. Much less frequently, only one alveolus is present. Sometimes, in very large glands, the excretory duct may branch; in this case there results a "compound" alveolar gland. In the Meibomian glands of the eyelids there is one long, straight duct, along the length of which there is a row of protruding alveoli.

The excretory ducts of sebaceous glands are lined by stratified squamous epithelium which is continuous with the external root sheath of the hair or with the Malpighian layer of the epidermis.

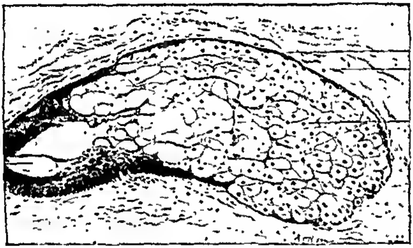

Fig. 290. Section of a human sebaceous gland. 120 ×.

The wall of the alveoli is formed by a basement membrance which is supported by a thin layer of fibrillar connective tissue. Along the internal surface is a single layer of thin cells with round nuclei. Toward the center of the alveoli a few cells cornify but most of them increase in size, become polyhedral, and gradually become filled with fat droplets. The central portion of the alveoli is filled with large, mutually compressed cells, whose cytoplasm is distended with fat droplets; after dissolution of the fat, these cells appear coarsely vacuolated in sections. In the very thin strands of cytoplasm between the vacuoles the remains of mitochondria are still to be seen. The nuclei gradually shrink and then disappear and the cells break down into fatty detritus which is mixed with horny scales. This is the oily secretion of the gland and is excreted either onto the hair or directly upon the surface of the epidermis.

In sebaceous glands, the secretion results from the destruction of the epithelial cells and is, therefore, of the *holocrine* type; it is followed by a regenerative multiplication of epithelial elements. In the body of the gland, mitoses are very rare in the cells lying on the basement membrane; they are numerous, however, in the cells close to the walls of the excretory ducts whence the new cells move into the secretory regions.

Sweat Glands. The sweat glands are distributed along the surface of the skin, with the exception of the margins of the lips, the glans penis, and the nail bed. They are simple, coiled, tubular glands; *i. e*, the secretory portion is a simple tube which is folded by several unequal twists into a ball, and the excretory duct is a narrow, unbranched tube (Fig. 291).

The mass of the secretory portion is located in the derma and measures 0.3 to 0.4 mm. in diameter. In the armpit and about the anus the bodies of some of the sweat glands may reach 3 to 5 mm. in

is penetrated by parallel fibrils which consist of a special "onychogenic" substance. On passing into the substance of the proximal edge of the nail plate, these cells lose their fibrillar structure, cornify, and become homogeneous.

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GLANDS OF THE SKIN

In man the glands of the skin include the sebaceous, sweat, and mammary glands. The latter are described in a separate chapter.

Sebaceous Glands. The sebaceous glands are scattered over the surface of the skin (except in the palms and soles). They lie in the derma and their excretory duct opens into the neck of a hair sac. When several glands are connected with one hair, they are closely placed and lie at the same level. On the lips, about the corners of the mouth, on the glans penis, and the internal fold of the prepuce, on the labia minora, and on the mammary papilla the sebaceous glands are independent of hairs and open directly on the surface of the skin; to this category also belong the Meibomian glands of the eyelids. The sebaceous glands in mucocutaneous junctions are more superficial than those associated with hairs.

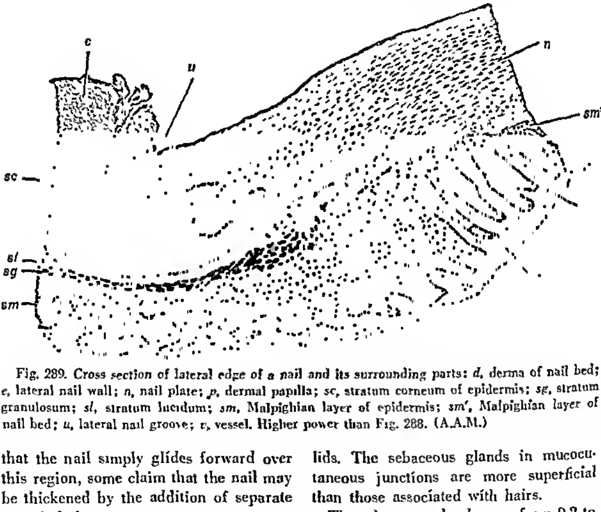

Fig. 289. *Cross section of lateral edge of a nail and its surrounding parts: d, derma of nail bed; c, lateral nail wall; n, nail plate; p, dermal papilla; sc, stratum corneum of epidermis; sg, stratum granulosum; sl, stratum lucidum; sm, Malpighian layer of epidermis; sm', Malpighian layer of nail bed; u, lateral nail groove; v, vessel. Higher power than Fig. 288.* (A.A.M.)

The sebaceous glands vary from 0.2 to 2 mm. in diameter; the largest, such as those on the nose, are not accessories of the hairs, but, on the contrary, each is provided with a very small hair.

The secretory portions of the sebaceous glands are rounded sacs (alveoli). As a rule, several adjacent alveoli form a mass like a bunch of grapes, and all of them open into a short, excretory duct; in this

slitlike or starlike shape, while the myoepithelial and glandular cells on the basement membrane are replaced by a double-layered, thin epithelium. The cells of the external layer have comparatively large nuclei and rather abundant mitochondria; the free surface of the cytoplasm of the cells of the internal layer is condensed and refractile.

In the epidermis the lumen of the excretory duct is devoid of a wall of its own and is simply an intercellular channel surrounded by concentrically arranged epidermal cells. The latter, in the Malpighian layer, have fine, keratohyalin granules in their cytoplasm (Fig. 293).

In certain parts of the skin the sweat glands have a peculiar arrangement and function. Such are the *glands which produce cerumen* in the external auditory meatus. They reach a considerable size and extend up to the perichondrium; their secretory portions branch and the excretory ducts, which sometimes also branch, may open together with the ducts of the adjacent sebaceous glands into the hair sacs of the fine hairs. In the terminal portions there are highly developed smooth muscle cells; the glandular cells which are located upon them are particularly rich in lipoid-containing pigment granules.

Moll's glands of the margin of the eyelid are also a special kind of sweat gland with terminal portions which do not form a ball, but are only irregularly twisted and are provided with a wide lumen. The excretory ducts open freely or into the hair sacs of the eyelashes.

The secretion of the various sweat glands is not everywhere alike; this is a reflection of the varying structure of the glands in different parts of the body. The true sweat, a transparent watery liquid, is excreted mainly by the small sweat glands, while those of the axilla and about the anus produce a thicker secretion of a complex, unknown composition. In women the apocrine sweat glands of the axilla show periodic changes with the menstrual cycle. These changes consist mainly in enlargement of the epithelial cells and of the lumens of the glands in the premenstrual period, followed by regressive changes during the period of menstruation (Fig. 294).

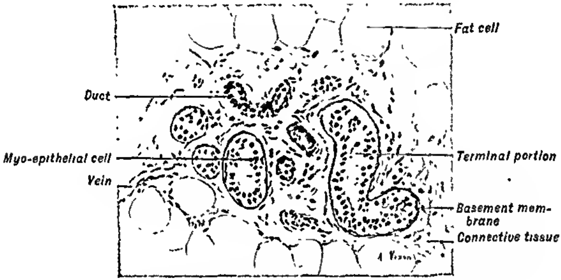

Fig 292. Section of a human sweat gland. 120 ×.

Blood and Lymphatic Vessels of the Skin. The arteries which supply the skin are located in the subcutaneous layer. Their branches, reaching upward, form a network (rete cutaneum) (Fig. 295, *R*) on the boundary line between the derma and the hypodermis; this is parallel to the surface. From one side of this network branches are given off which nourish the subcutaneous stratum with its fat cells, sweat glands, and the deeper portions of the hair sacs. From the other side of this network vessels enter the derma; at the boundary between the papillary and reticular layers they form the denser, subpapillary network or the rete subpapillare (Fig. 295,

diameter. In these regions they are red and are located deep in the subcutaneous layer.

At the transition of the secretory portion into the excretory duct the tube suddenly becomes thin. Coming toward the surface, through the derma, the duct is slightly twisted and curved In passing through the Malpighian layer and in particular through the horny layer of the epidermis, it is spirally twisted. On the palms and soles and on the ventral surface of the fingers, the rows of ducts open on the ridges of the external pattern with funnel-shaped openings which can be seen easily with a magnifying glass.

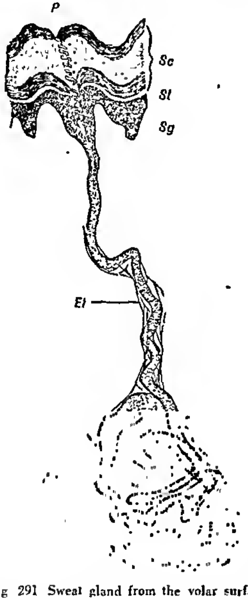

Fig 291 Sweat gland from the volar surface of an index finger. The drawing was made from a combined study of sections and a teased preparation: *P*, Sweat pore; *Sc*, stratum corneum; *Sl*, stratum lucidum; *Sg*, stratum germinativum; *El*, elastic tissue surrounding the duct; *Amp*, ampulla; *D*, sudoriferous duct. 45 ×. Slightly modified after v. Brunn.

The walls of the secretory portion are composed of a thick basement membrane. Externally it is supported by a slightly thickened layer of connective tissue. Directly inside the basement membrane are flattened spindle-shaped cells 30 to 90 μ in length, with their long axis parallel or slightly tangential to that of the glandular tube. They contain an elongated nucleus, and the rest of the cell is filled with longitudinal fibrils. It is supposed that these "myo-epithelial" cells, by contracting, help to discharge the secretion. They are particularly numerous and are highly developed in the large sweat glands of the axillary and perianal regions.

The glandular cells which excrete sweat have a cubical or truncated pyramidal form and are located in one layer upon the myo-epithelial cells in such a fashion that their bases enter the spaces between them. At the base is a rather large round nucleus; the cytoplasm contains mitochondria and, near the lumen of the duct, a varying number of secretory granules and vacuoles, depending on the functional state of the cell. Sometimes there are also fat droplets, glycogen and pigment granules. The presence of pigment explains why the secretion of certain sweat glands, as the axillary, appears stained. The free surface of the cells often shows protrusions of protoplasm, as constricted buds, which were believed by some to separate and become a part of the secretion (apocrine glands).

Between these glandular cells there are typical secretory capillaries. The caliber and the shape of the free lumen of the secretory portion fluctuate greatly with the functional state of the gland.

The glandular tube in passing over into the excretory duct suddenly becomes much narrower, the lumen acquires a simple,

derm, while the derma arises from the mesenchyme.

Epidermis. The epidermis in the human embryo, during the first two months, is a double-layered epithelium. The basal layer which lies on the mesenchyme consists of cuboidal or cylindrical cells which multiply energetically. The peripheral layer consists of flat cells which are constantly formed anew from the elements of the deeper layer.

The irregularities on the lower surface of the epidermis arise at the end of the third month on the inner surfaces of the fingers, palms, and soles as parallel ridges protruding into the derma; from the beginning they show a characteristic pattern. From them sweat glands develop. Protruding, longitudinal cushions corresponding to the ridges are formed on the external free surface of the epidermis.

Derma. The derma and hypodermis consist during the first month and a half of mesenchyme with wandering cells. From the second month on, the fibrillar interstitial substance appears. Elastic fibers appear later. In still later stages, the mesenchyme divides into a peripheral dense layer with a compact arrangement of its elements—the derma—and the deep loose layer, the future subcutaneous layer. In the derma, in turn, the peripheral papillary layer differentiates.

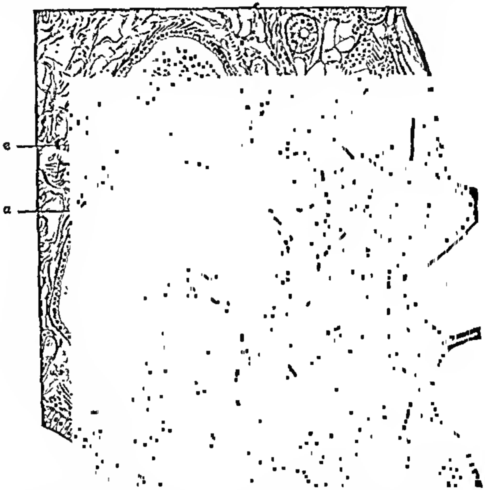

Fig. 294. Axillary glands, from a woman of thirty-seven years, during the premenstruum: *a*, Greatly enlarged glands which change with the menstrual cycle and, e, glands which do not change. Resorcin-fuchsin stain for elastic fibers. (Preparation of Loescke.) 110 ×. After Hoepke.

Beginning with the third month, the epidermis becomes three layered. The new intermediate layer above the basal cells consists of polygonal cells which increase in number and become interconnected by intercellular bridges. At the end of the third month, in the peripheral portions of the intermediate layer, cornification begins and leads to the formation of the layers as found in the adult. The horny scales are desquamated and form part of the vernix caseosa. Pigment granules, even in heavily pigmented races, usually appear in the deep cells of the Malpighian layer only after birth.

Hair. In man, hair first appears in the eyebrows and on the chin and upper lip, at the end of the second month. At first, in the deep layer of the epidermis, a group of cylindrical,

Rp). This gives off thin branches which enter the papillae and form networks inside them.

The veins which collect the blood from the capillaries in the papillae form the first network of very thin veins immediately beneath the papil-

Fig. 293. Section through the skin from the head of a man of twenty-two years. The end of the excretory duct of a sweat gland· *E*, Eleidin; *K*, keratohyalin; *D*, degenerating cells; *V*, hypertrophic cells. 600 ×. Drawing by Vierling, after Hoepke.

lae. Then follow three flat networks of gradually *enlarging* veins on the boundary line between the papillary and reticular layers. In the middle section of the derma and also at the boundary between the derma and the subcutaneous tissue, the venous network *is on the same level* as the arterial rete cutaneum. Into this network the veins of the sebaceous and the sweat glands enter. From the deeper network the large, *independent*, subcutaneous veins pass, as well as the deep veins accompanying the arteries.

Each hair sac has its own blood vessels. It is supplied with blood from three sources: From a special small artery which gives off a capillary network into the papilla, from the rete subpapillare toward the *sides of the hair* sac, and from several other small arteries which form a dense capillary network in the connective tissue layer of the follicle.

There is a dense network of capillaries outside the basement membrane of the sebaceous and, in particular, of the sweat glands.

The skin is rich in lymphatic vessels. In the papillary layer they form a dense, flat meshwork of lymphatic capillaries. They begin in the papillae as networks or blind outgrowths which are always deeper than the blood vessels. From this *peripheral* network branches pass to the deeper network which lies on the boundary between the *derma* and the hypodermis, under the rete cutaneum; it has much wider meshes and its vessels are provided with valves. From the deeper network large, subcutaneous lymphatic vessels originate and follow the blood vessels. Lymphatic vessels are not connected with the hairs or the glands of the skin.

Nerves of the Skin. The skin, with its accessories, serves as an organ for receiving impulses from the external environment; it is accordingly abundantly supplied with sensory nerves. In addition, it contains nerves *which supply the blood* vessels, muscles, etc.

In the subcutaneous stratum are rather thick nerve bundles which form networks composed mainly of myelinated and some nonmyelinated fibers. The branches which are given off by this reticulum form, in the derma, several new thin plexuses. Among them the network on the boundary between the reticular and papillary layers stands out clearly as does also the subepithelial one.

In all the layers of the *hypodermis, derma,* and epidermis are many different *kinds of nerve* endings. These are discussed *in the* section on the Nerve Endings. Among *them, the sensory* endings probably are all *connected with* the craniospinal myelinated fibers; the nonmyelinated fibers lead to the blood vessels, smooth muscles and glands. The abundant nerves of the hair undoubtedly play an *important part* in the reception of tactile *stimuli*.

Histogenesis of the Skin and of Its Accessories. The *epidermis* develops from the ecto-

fifth month, and without the participation of keratohyalin, in the portion of the nail bed near the proximal nail groove. Here the deep layer of the epidermis is transformed into the nail matrix and its cells are penetrated by the fibrils of onychogenic substance; they become flat, adjoin one another closely, and give rise to the true nail plate. In the beginning it is still thin and is entirely buried in the epidermis of the nail field or bed. It gradually moves in the distal direction. The layers of epidermis which cover the plate eventually desquamate.

Sweat Glands The development of the sweat glands in man proceeds independently of the hairs in most places of the skin The first primordia appear during the fifth month on the palms and soles and the lower surface of the fingers. At first they are similar to the primordia of the hairs. An epithelial shaft with a terminal thickening grows into the underlying connective tissue. But unlike that about the hairs, the connective tissue here does not condense about the epithelium The shaft gradually elongates, becomes cylindrical, and its lower portion curls in the form of a ball. Beginning with the seventh month an irregular lumen forms in this lower portion which constitutes the secretory part; along the course of the future excretory duct another lumen develops and later unites with the former. In the secretory portion the epithelium around the lumen forms two layers, which differentiate into an external layer of myo-epithelial elements and into an internal layer of glandular cells.

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dividing cells appear. These grow into the underlying connective tissue and produce a gradually elongating, epithelial cylinder. This is the primordium of a hair follicle, the so-called "hair germ"; it is rounded and slightly flattened on its end. Under the latter an accumulation of condensed connective tissue appears very early. From it the hair papilla forms and protrudes into the in which Henle's layer is the first to appear. The mass of the cells on top of the papilla represents the primordium of the shaft itself and becomes cornified a little later. The layer of epithelium which remains on the outside of the sheath of Henle becomes the external root sheath. The shaft of the new hair elongates, due to the multiplication of cells of the matrix on the summit

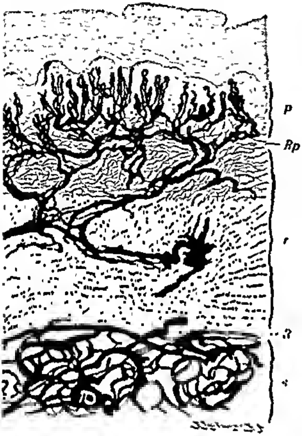

Fig. 295. Distribution of blood vessels in the skin: s, Subcutaneous tissue; r, reticular layer of derma; p, papillary layer of derma; R, rete cutaneum; Rp, rete subpapillare. Modified slightly after v. Brunn.

Fig. 296. Two early hair primordia of the frontal skin of a three months' embryo 1: First primordium; G, border of the derma; HC, hair canal cells; HK, hair germ; P, primordium of the papilla; 2: slightly later stage, HB, primordium of the dermal hair sheath; W, external root sheath. 740 ×. After Schaffer.

epithelial mass of the bulb (or germ). The epithelial cells at the surface of the connective *tissue papilla represent the matrix* of the future hair. The connective tissue which surrounds the bulb later forms the *connective tissue portions* of the hair sac. On the surface of the epithelial hair bulb, two projections arise; the upper represents the primordium of the sebaceous gland; its central cells early *undergo a fatty transformation.* The lower protuberance is located at the place of attachment to the hair sac of the arrector pili muscle.

In the mass of the epithelium which forms the hair primordium, there differentiates a layer of rapidly cornifying cells. This layer has the shape of a hollow cone open toward the papilla; it is the primordium of the internal root sheath, of the papilla and perforates the top of the hollow cone of Henle's sheath. The tip of the hair moves upward, pierces the epidermis and protrudes above the surface of the skin.

Nails. The development of the nails begins in the third month by the formation, on the back of the terminal phalanx of each finger, of a flat area, the *primary nail field.* This is surrounded by a fold of the skin. In the region of the nail the epithelium has three or four layers. The true nail substance is laid down during the

cera to move freely in the cavity. The wall of the digestive tube is richly provided with blood vessels which bring nutritive materials and oxygen as well as the raw materials necessary for the secretory activity. These vessels carry a large part of the absorbed products of digestion from the mucous membrane of the small intestine to the rest of the body. The rest of the absorbed products enter the lymphatics of the intestines. The wall of the digestive tract contains an intricate system of sympathetic nervous ganglia and plexuses which regulate the movements of the tube.

THE ORAL CAVITY

The mucous membrane in the mouth is similar to the skin. The epithelium is of the stratified squamous type and in its deeper layers is more or less distinctly fibrillated. In man, under physiologic conditions, it does not undergo cornification. The nucleus of the cells of the superficial layers shrinks and degenerates, but does not disappear, and the cell body does not reach the same degree of flatness as in the epidermis. These superficial cells are always shed in large quantities and are found in the saliva. In some places they contain granules of keratohyalin. In the

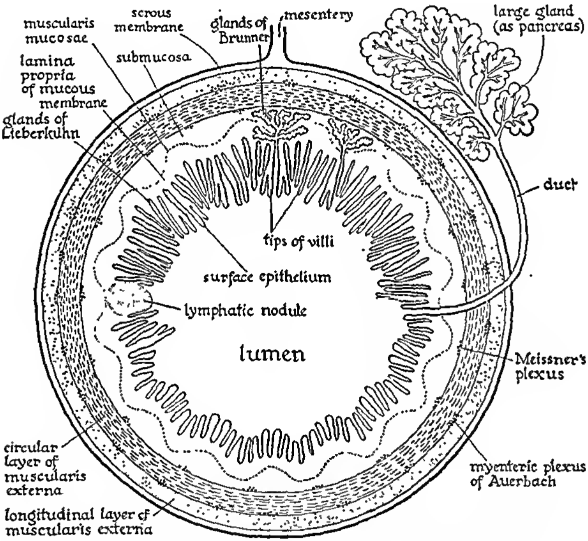

Fig. 297. Diagram of cross section of intestinal tract. In the upper half of the drawing the mucous membrane is provided with glands and villi, in the lower half it contains only glands.

Chapter XVI

THE ORAL CAVITY AND ASSOCIATED STRUCTURES

GENERAL REMARKS ON THE DIGESTIVE SYSTEM

THE digestive system is a long, winding tube which begins with the lips and ends with the anus. On its way through this tract the food undergoes complex mechanical and chemical changes. It is minced and ground by the teeth, is forwarded through the tube by the contraction of its muscular walls, and is digested by the secretions of the various parts of the alimentary system and its auxiliary glands. A part of the digested food is absorbed by the walls of the intestine and passes into the circulation which carries it into the tissues of the organism; the residue is eliminated as feces.

The digestive tract consists of the following successive parts: Mouth, pharynx, esophagus, stomach, small intestine, large intestine, and rectum. The functional condition of one segment causes certain functional changes in the following and thus the regular sequence of the processes necessary for the digestion of food is assured.

In the embryo the entoderm is transformed into the epithelial structures of the alimentary canal; the visceral mesoderm gives rise to its connective and muscular tissues. In the adult the inner surface of the wall of the digestive tube is lined throughout by a *mucous membrane.* It consists of a superficial layer of epithelium and of a layer of connective tissue, the *lamina propria.* The wall of the tube contains smooth muscles which form the *muscularis externa.* (See Fig. 297).

In most parts of the digestive tube the outer limit of the mucous membrane is marked by a thin, muscular layer, the *muscularis mucosae.* Between it and the muscularis externa, there is a layer of loose connective tissue, the *tela submucosa.* Where the muscularis mucosae is absent, the lamina propria gradually passes into the submucosa.

In the adult the mucous membrane forms numerous outgrowths which increase the surface of the epithelium. The mucous membrane of the mouth forms the teeth. The mucous membrane is provided with many invaginations, the *glands* or *crypts.* They are lined by epithelium which continues into them from the surface; some of them elaborate liquids which split the food into its simple chemical constituents—digestion—while others produce mucus which lubricates the surface of the mucous membrane. Some of the glands remain confined to the thickness of the mucous membrane. Others grow to such an extent that they become separate organs, connected with the epithelial surface from which they originated by long excretory ducts. In the oral cavity, esophagus and rectum, the wall of the digestive tube is surrounded by a layer of dense connective tissue which attaches it to the neighboring organs. The outer surface of the stomach and intestines, which are suspended in the peritoneal cavity by the mesenteries, is covered with a serous membrane which permits these vis-

brane can be easily lifted into folds. In those places against which the food is crushed and rubbed, as on the hard palate, there is no submucosa, and the mucous membrane is firmly connected with the underlying periosteum or muscles.

The inner zone of the lip margin in the newborn is considerably thickened and is covered with hairless sebaceous glands and contains many high papillae. This seems to facilitate the process of sucking. Into these free papillae project the labial glands. The labial mucous membrane contains small, mixed glands (see below).

The *soft palate* consists of layers of striated muscle and fibrous tissue on both surfaces and on the posterior margin. It is covered with a mucous membrane. On the oral surface the latter has the structure typical of the oral cavity—a stratified squamous epithelium, high interepithelial papillae, and glands of the pure mucous type. These are surrounded by adipose tissue and are scattered in a loose submucous layer separated from the lamina propria by dense elastic networks. This oral type of mucous membrane also covers the posterior margin of the soft palate and continues upon the nasal surface. On this surface, at varying distances from the margin, the stratified epithelium is substituted by pseudostratified, ciliated columnar epithelium ("respiratory epithelium") which rests on a thickened basement membrane. The lamina propria contains small glands of the mixed type, but no adipose tissue, and is infiltrated with lymphocytes. A dense layer of elastic fibers is found between the glands and the muscles. A submucosa is not present (Fig. 299).

THE TONGUE

The tongue consists of interlacing bundles of striated muscle which run in three planes and cross one another at right angles; the muscular mass is covered by a tightly adherent, mucous membrane. The dense lamina propria is fused with the interstitial connective tissue of the muscle and a submucous layer is present only on the under surface. While the lower surface of the tongue is smooth, the dorsal surface is uneven and shows a striking difference in its anterior part—the body—and in its smaller, posterior part—the base or root of the tongue. In the first the mucous membrane is covered by a multitude of small excrescences of varying form—the *papillae;* in the second it presents only irregular bulgings. The boundary line between the two regions is V-shaped, with the opening of the angle directed forward. This is the gustatory region of the tongue. At the head of the angle is a small invagination of the surface, the *foramen caecum. It is the rudiment of the thyroglossal duct which in early embryonic stages connects the thy-*

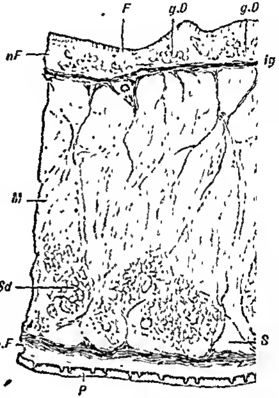

Fig. 299. Portion of a sagittal section through the soft palate of a girl of nine years: *nF*, Nasal surface; *F*, ciliated epithelium with goblet cells; *g.D*, mixed glands; *ig*, infraglandular layer of elastic fibers; *M*, musculature; o *F*, oral surface; *P*, stratified squamous epithelium with papillae; *Sd*, mucous glands; *S*, submucosa. Resorcin-fuchsin stain. 12 ×. After Schumacher.

cells of the middle and superficial layers there is usually some glycogen. In many animals the epithelium of the oral cavity undergoes extensive cornification.

The *lamina propria* is provided, in most places, with papillae similar to those of the *skin*. The structure is, however, more delicate, and the collagenous and elastic fibers thinner than in the derma. In the posterior section of the oral cavity, it contains many lymphocytes which are often found migrating into and through the epithelium. The arrangement of the blood vessels is similar to that of the skin. There is a deep submucous plexus of large vessels, from which branches arise and form a second plexus in the lamina propria, which in turn sends small branches into the papillae. The lymphatics also show an arrangement similar to that in the skin, and begin with blind capillary outgrowths in the papillae.

The *oral mucous membrane* is very sensitive and is provided with many nerves belonging to the sensory branches of the trigeminal nerve (lingual nerve). It also contains the specific end organs of the sense of taste (p. 357).

In most places under the lamina propria, especially in the cheeks, and on the soft palate, there is a fat-containing, loose *submucosa* into which the dense connective tissue of the mucosa gradually merges. In such places the mucous mem-

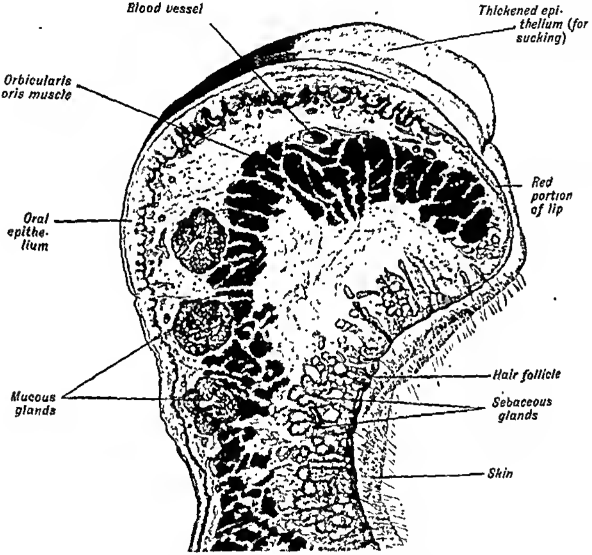

Fig 298. Camera lucida drawing of sagittal section through lip of newborn infant. Stained with hematoxylin. 10 ×. Drawn by Miss E. Bohlman.

roid gland primordium with the surface epithelium of the oral cavity.

Papillae. Three types of papillae are present on the body of the tongue: (1) The filiform, (2) the fungiform, and (3) the circumvallate. The first are arranged in more or less distinct rows diverging to the right and left from the middle line and parallel to the V-shaped gustatory region. The fungiform papillae are scattered singly between the filiform and are especially numerous near the end of the tongue. The circumvallate papillae, numbering 10 to 12 in man, are arranged along the gustatory lines.

The *filiform papillae* are 2 to 3 mm. long. Their core is a connective tissue ridge beset with numerous, secondary papillae with pointed ends. The epithelium which covers these connective tissue outgrowths follows their outlines and also forms short, secondary papillae which taper at their ends into long pointed processes (Fig. 301). In man the superficial squamous cells are transformed into hard scales containing shrunken nuclei. Their substance, however, is not true keratin. While the axial parts of the scales at the point of the papilla are connected with its solid axial strand, their lower edges project from the surface of the papilla in the fashion of the branches of a fir tree. When digestion is disturbed the normal shedding of these scales is delayed and they accumulate, in layers mixed with bacteria, on the surface of the tongue which thus is covered with a gray film—the "coated" tongue.

The *fungiform papillae* reach a height of 0.7 to 1.8 mm. and a thickness of 0.4 to 1 mm.; they have a short, slightly constricted stalk and a spherical, slightly flattened, upper part. The connective tissue core forms many secondary papillae; the epithelium covering them has a smooth free surface (Fig. 302). On many, but not all, of the fungiform papillae the epithelium contains taste buds in the secondary papillae. As the core is rich in blood vessels, the fungiform papillae have a marked red color.

The *circumvallate papillae* are sunk into the surface of the mucous membrane and each is surrounded by a deep, circular furrow 1 to 3 mm. in diameter. In a perpendicular section the papilla has an inverted conical form. The connective tissue core forms secondary papillae only on the upper surface. The covering epithelium is

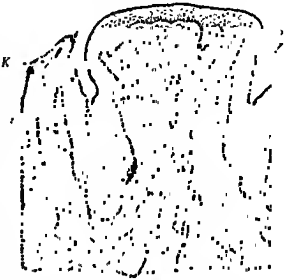

Fig. 302. Perpendicular section through a fungiform papilla from the tongue of a man: *A*, Artery; *B*, connective tissue stroma of the fungiform papilla; *KH*, keratohyalin granules in the superficial cells of a filiform papilla; *M*, cross striated muscle fibers; *N*, nerve; *P*, secondary papillae; *V*, vein; *Z*, cellular stroma with lymphatic and blood vessels. 46 ×. After Schaffer, from Schumacher.

smooth while that of the lateral surfaces of the papillae contains many taste buds (Figs. 303, 305).

In a vertical section, 10 to 12 of them can be seen on the lateral surface of the papilla. In the outer wall of the circular groove surrounding the papilla a few taste buds may be present. The number of taste buds in a single papilla is subject to great individual variations. On the average it has been estimated at 250.

Connected with the circumvallate papillae are glands of albuminous type (*glands*

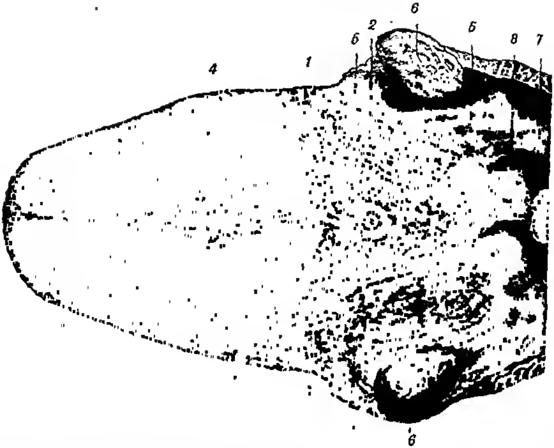

Fig. 300. The surface of a human tongue: *1* and *2*, Vallate papillae; *3*, fungiform papillae; *4*, rows of filiform papillae; *5*, lingual tonsils; *6*, palatine tonsils; *7* epiglottis; *8*, median glosso-epiglottic fold. After Sappey, from Schumacher.

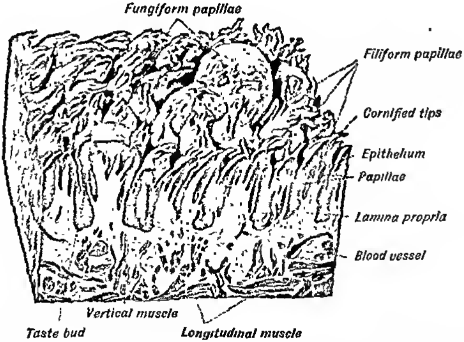

Fig. 301. Surface of the dorsum of the tongue, drawn through a combined study with the binocular microscope and of sections. The anterior cut surface corresponds with the long axis of the tongue—the tip of the tongue being to the reader's left. 16 X. After Braus.

of v. Ebner) whose bodies are embedded deep in the underlying muscular tissue and whose excretory ducts open into the bottom of the furrow (p. 364). The connective tissue of the papillae and of the outer wall of the furrow sometimes contains a circular bundle of smooth muscles and accumulations of lymphocytes, especially around the secretory ducts of the glands of v. Ebner.

On the lateral surface of the posterior part of the tongue the paired *foliate papillae* may be found. In man they are rudimentary, while in many animals they are highly developed and represent the main peripheral organ of taste. The fully developed foliate papillae, as found on the tongue of the rabbit, are longitudinal, oval bulgings on the mucous membrane. They consist of many parallel, transversely arranged ridges with deep grooves between them. The epithelium of the lateral surfaces of the ridges, which face each other, contains many taste buds. Small albuminous glands open into the bottom of the furrows between the ridges

In addition to those on the surface of the tongue, taste buds are found on the glossopalatine arch, on the soft palate, on the posterior surface of the epiglottis, and on the posterior wall of the pharynx down to the level of the inferior edge of the cricoid cartilage In the newborn their number is larger than in the adult. The upper free surface of the circumvallate papillae carries them in the newborn, although they disappear from this place in later life.

The bulgings on the root of the tongue are caused by peripheral lymphatic nodules, the *lingual tonsils*, and the *follicles of the tongue* (Fig. 304). On the free surface of each lingual tonsil a small opening can be noticed. It leads into a deep, irregular invagination lined with stratified squamous epithelium—the *crypt*. The epithelium of the crypt is surrounded by lymphatic tissue; innumerable lymphocytes infiltrate the epithelium and assemble in the lumen of the crypt, where they degenerate and form masses of detritus with the desquamated epithelial cells and bacteria. The lingual tonsils are often connected with small glands of the pure mucous type whose secretory portions are embedded in the underlying muscle tissue, while their excretory ducts open into the crypt or on the free surface.

Taste Buds. The taste buds have the form of a barrel or a flask with a wide bottom and a short neck. Under low power they are seen in sections as pale, oval bodies in the darker stained epithelium. Their long axis measures about 72 μ. They stand upright in the epithelial layer and extend almost through its whole thickness, from the basement membrane to the surface. The superficial layer of squamous epithelial cells over each taste bud is pierced by a small opening—the *outer taste pore*.

Two cell types are usually distinguished among the constituents of a taste bud—the *supporting cells* and the *neuro-epithelial taste cells*. Among the former, periph-

Fig. 305. Two taste buds from side of circumvallate papilla of Macacus rhesus, showing taste pores at *P*. Drawn by Miss E. Bohlman

eral and central cells are described. The first are thick, spindle-shaped cells slightly flattened transversely and resembling a slice of a melon. They are connected with one another by their sides and thus constitute the thick wall of the barrel. Toward the upper pole of the bud they taper down and their ends surround a small opening, the *inner taste pore*, which lies beneath the outer pore and leads into a pitlike excavation. The walls of the latter are formed by the free ends of the central supporting cells which have a more slender form and are shorter the nearer they stand to the axis of the bud. They end at a lower level than the per-

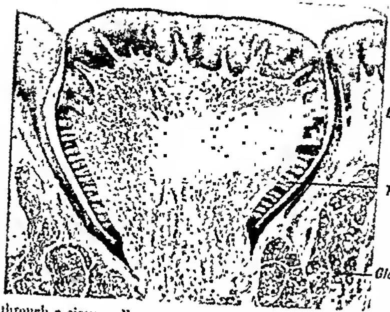

Fig. 303. Section through a circumvallate papilla of *Macacus rhesus*. Photomicrograph 42 ×.

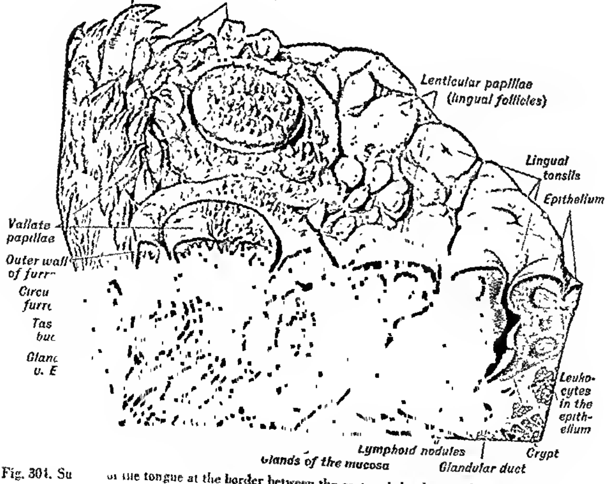

Fig. 304. Su of the tongue at the border between the root and the dorsum. Prepared as Fig. 301. 16 ×. After Braus

minous," watery liquid which lacks mucus, but contains salts, proteins, and ptyalin. In the mixed glands—containing serous and mucous cells—the secretion is a more or less viscid liquid containing mucin, salts, and ptyalin.

All glands of the oral cavity possess a system of branching excretory ducts. The secretory portions in the pure mucous glands are usually long, branching tubules. In the pure albuminous and mixed glands the secretory portions vary from oval acorns to tubulo-acinar forms provided with irregular outpocketings.

The initial intralobular ducts are thin, branched tubules called the necks or isthmuses or intercalated ducts. The next larger order of branches, also located in the interior of the smallest lobules, has a peculiar striated epithelium; these ducts are called "striated" (or "salivary") tubules. Then follow the larger branches; among them (in the large glands) lobular, sublobular, interlobular, and primary ducts may be distinguished.

Mucous Cells. In the pure mucous glands the mucous cells are arranged in a layer against the basement membrane and have an irregularly cuboidal form. In fresh condition their cytoplasm contains many round, pale droplets of mucigen, the antecedent of mucin, while the nucleus is invisible. In fixed and stained sections the droplets of mucigen are usually destroyed so that the cell body appears clear and contains an artificial network of cytoplasm and precipitated mucigen. This network stains in the manner which is typical of mucin, that is, red with mucicarmine, or metachromatically purple with blue, basic aniline dyes like thionin. The nucleus is at the base of the cell and usually appears angular and compressed by the accumulation of mucigen. The cytocentrum occupies the middle of the pale cell body; between the droplets of mucigen a few mitochondria and fragments of a Golgi net can be found. The free surface of the mucous cells is usually provided with a delicate network of terminal bars. Secretory canaliculi are absent. Usually the lumen of the terminal portions is large and filled with masses of mucin.

When the secretion leaves the cell it collapses, its cytoplasm increases relatively in amount and only a few granules of mucigen may remain confined to its free surface. The nucleus rises from the base of the cell and becomes round. In this condition the mucous cells may be mistaken for albuminous cells. The ab-

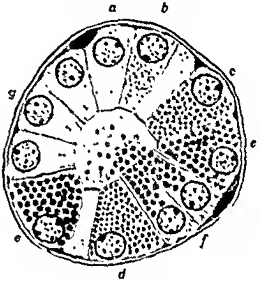

Fig. 306. Albuminous gland of a vallate papilla of a nineteen-year-old man, showing different functional states. The secretory cycle is indicated by the letters *a* to *g*. Centrioles in three cells. Basal cells are seen between the secreting cells and the basement membrane. Iron hematoxylin stain. After Zimmermann.

sence of secretory capillaries always distinguishes them from albuminous cells. The demonstration of these capillaries requires special staining methods. Under physiologic conditions, the mucous cells rarely discharge all of their granules.

The most reliable criterion for separating mucous and serous cells is the positive staining of mucus in the former. The staining reactions of the mucin elaborated by different mucous cells is not the same even in the same gland. Sometimes the mucous cell contains fat droplets. The mucous cells, as a rule, do not show any

ipheral cells. Inside of the bud, between the supporting cells, the taste cells are distributed. Their number in a taste bud has been estimated at 4 to 20. They have a slender, rod-shaped form with a nucleus in the middle and on the free surface a short, hairlike process, the *taste hair,* which projects freely into the lumen of the pit. The lower end of these cells terminates in a small rounded knob.

Nerves. The anterior two thirds of the tongue is innervated by the lingual nerve, which contains trigeminal fibers of general sensibility and facialis fibers of gustatory sensibility. The latter enter the lingual nerve from the chorda tympani. The posterior third of the tongue is innervated by the glossopharyngeal nerve for both general and gustatory sensibility. Taste buds of the epiglottis and lower pharynx are innervated by the vagus. These nerve fibers are lightly myelinated. They branch profusely under the basement membrane, lose their myelin, and form a subepithelial plexus, from which fibers penetrate the epithelium. Some terminate as intergemmal fibers *by free arborization between the taste buds;* others, the perigemmal fibers, closely envelop the taste buds; and still others, the intragemmal fibers, penetrate the taste buds and end with small terminal enlargements in intimate contact with the taste cells. The functional significance of these different nerve endings is unknown.

Histophysiologic Remarks. Physiologic analysis shows that only four fundamental varieties of taste sensations exist —sweet, bitter, acid, and salty. It has been shown by the application of substances to individual fungiform papillae that they differ widely in their receptive properties. Some do not give any taste sensations, while others give sensations of one or more taste qualities. No structural differences in the various taste buds have been found, in spite of the differences in sensation mediated. There is, moreover, a general chemical sensitivity in regions of the mouth where there are no taste buds.

GLANDS OF THE ORAL CAVITY

General Description. Numerous *salivary glands* open into the oral cavity. Many of these are small glands in the mucosa or submucosa and have special names according to their location. They seem to *secrete continuously* and furnish a liquid, the *saliva,* which moistens and lubricates the oral mucous membrane. In addition to these there are three pairs of large glands which constitute the salivary glands proper. They are the *parotid,* the *mandibular (submaxillary),* and the *sublingual* glands. They secrete only when mechanical, thermal or chemical stimuli act upon the nerve endings in the oral mucous membrane, and as the result of certain psychic or olfactory stimuli. The saliva secreted by the large glands may be very abundant and helps prepare the food for digestion in the stomach and intestine.

The *saliva* collected from the oral cavity is a mixture of the secretions of the various salivary glands. It is a viscous, colorless, opalescent liquid which contains water, mucin, some proteins, mineral salts, and an enzyme (ptyalin) which splits starch into water-soluble, less complex carbohydrates. Saliva always contains a varying number of desquamated squamous epithelial cells and salivary corpuscles; most of the latter originate in the follicles of the tongue and in the tonsils and are degenerated lymphocytes or granulocytes.

The quality of the saliva collected from the oral cavity varies with the predominant participation of one or the other of the glands in its formation. But even the secretion of one gland may change considerably with variations in the stimuli acting upon the oral mucous membrane, as, for instance, with different kinds of food.

These glands may be classified in three categories according to the type of their secretory cells. The glands containing only *mucous* cells elaborate a viscid secretion which consists almost exclusively of mucin. In glands with only *albuminous* cells the secretion is a "serous" or "albu-

bined into one group and given a general name because histologic methods are not sensitive enough to make the differences visible microscopically. In many cases their secretory granules give a more or less distinct staining reaction for mucus with mucicarmine; such cells are called "muco-albuminous" or "mucoserous" (also "trophochrome").

the albuminous cells predominate, some of the terminal portions may be exclusively albuminous. In others a part of the secretory portion is lined with mucous, and a part with serous cells. In sections the mucous portions can often be recognized by their clear aspect but more certainly by their color after specific staining of the mucus.

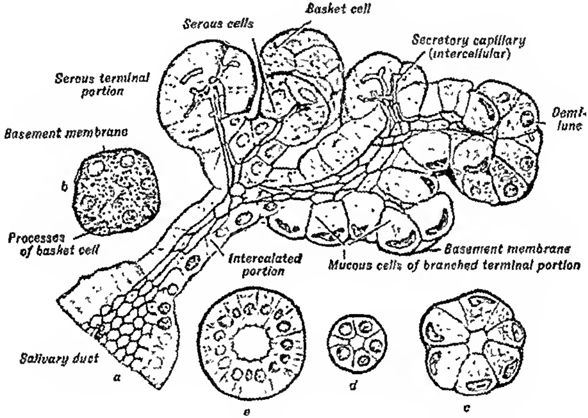

Fig 308. Reconstruction of a terminal portion and its duct of a submaxillary gland: *a*, Section through the whole model, *b*, cross section of a purely serous terminal portion, showing basal lamellae; *c*, cross section through a purely mucous terminal portion, *d*, cross section through an intercalated portion; *e*, cross section through a salivary duct Redrawn and modified after a reconstruction by Vierling, from Braus.

The Cells in the Mixed Glands. The relative number of the two kinds of glandular cells in the mixed glands varies within wide limits. In some cases the albuminous cells are far more numerous than the mucous cells, while in other cases the reverse is true: in still other instances both cell types are present in about equal numbers. The mucous and albuminous cells line different parts of the terminal portion. In those mixed glands in which

As a rule, the mucous cells are near the excretory ducts, while the serous cells are confined to the blind end of the secretory portion. It is quite probable that the mucous cells in mixed glands arise through the mucous transformation of the cells in the smallest excretory ducts, the *necks* or *isthmuses* Sometimes single mucous cells are scattered between the unchanged cells of the isthmus. In other cases the part of the isthmus directly adjoining the ter-

signs of degeneration; mitoses have occasionally been observed in them.

Albuminous Cells. These elements, when filled with secretion in a resting gland, in fresh condition, contain a multitude of small, highly refractile, *secretion granules*, in a homogeneous cytoplasm. The cell boundaries are not distinct. The roughly cuboidal cells surround a small tubular lumen.

The secretory granules of the albuminous cells accumulate between the nucleus and the free surface. After the gland has secreted for a certain time the albuminous cells diminish in size; their granules become less numerous and are confined to the free surface of the cells. In extreme, nonphysiologic cases, all of the granules may disappear. As the albuminous cells are probably the source of ptyalin, the granules are to be looked upon as zymogen granules, the antecedents of the enzyme. Before leaving the cell they are transformed into secretory vacuoles.

In cells crowded with secretion the nucleus is spherical, small and darkly staining; it occupies a position at the base of the cell and may show irregular indentations. Besides the secretory granules, the cytoplasm contains rod-shaped mitochondria and a Golgi net above the nucleus. A cytocentrum near the free surface is distinct only in empty cells.

At the base of the cell around the nucleus, and sometimes above it, is an accumulation of chromophil substance, apparently ribose nucleoprotein, which stains darkly with basic dyes and, owing to its arrangement in parallel lamellae, causes a vertical striation. By appropriate technic mitochondria can be demonstrated between the lamellae. The albuminous cells often contain fat or glycogen. On their free surfaces the albuminous cells are provided with a system of terminal bars; between their lateral surfaces there are always secretory capillaries. Mitoses occur occasionally.

The albuminous cells of the different glands of the mouth are not identical functionally, although they may seem to have the same structure. They are com-

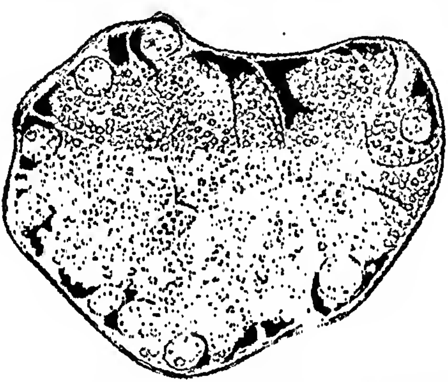

Fig. 307. Acinus of a human submaxillary gland showing chromophil substance (black) and zymogen granules Acid fuchsin and toluidine blue stain. 1610 ×. Courtesy of R. R. Bensley.

and calcium salts to the secretion. These tubules (as well as the larger ducts) sometimes present a succession of constricted and dilated sections. In the larger ducts the epithelium is columnar, pseudostratified, and occasionally contains goblet cells. Nearing the opening on the mucous membrane, it becomes stratified for a short stretch and is then succeeded by stratified squamous epithelium.

Fig. 311. Section through the parotid gland of a man: *a*, Artery; *F*, fat cell; *IB*, interlobular connective tissue; *S*, intercalated duct; *SA*, secreting terminal portion; *SP*, branching salivary duct. Mallory's connective tissue stain. 110 ×. After Schaffer.

CLASSIFICATION OF THE ORAL GLANDS BY THEIR LOCATION

A. Glands which open into the vestibule of the mouth:
   1. Parotid gland, with a duct opening into the vestibule.
   2. Labial glands, scattered in the mucous membrane of the upper and lower lips.
   3. Buccal glands, a continuation of the labial glands in the mucous membrane of the cheek.

B. Glands which open on the bottom of the oral cavity, between the tongue and the mandible:
   1. Mandibular (submaxillary) gland—a large gland with a duct opening at the side of the frenulum of the tongue.
   2. Sublingual glands, situated beneath the mucous membrane at the side of the frenulum of the tongue. Among them:
      (*a*) The large sublingual gland with a duct opening into the duct of the mandibular gland.
      (*b*) Several small glands varying in number and size. Their ducts open in many places along a fold of the mucous membrane, the plica sublingualis. At the posterior end of this group are the small glossopalatine glands.

minal portion is lined exclusively with mucous cells, so that only a small, proximal part of the isthmus remains unchanged. If the mucous transformation affects all the cells in the neck, this part of the system of the excretory ducts ceases to exist as such and the mucous cells adjoin the striated tubules. If the mucous cells are not very numerous, the secretory portion of the gland will show an irregular mixture of pale mucous, and dark albuminous cells. Often both cell types may be seen lining different parts of the wall of the same cavity.

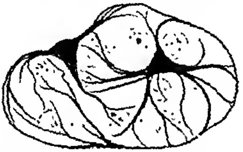

Fig. 309. Branching basal (basket or *myo-epithelial*) cells with dark *fibrils*; *from* an albuminous *terminal* portion of a human submaxillary gland. Iron-hematoxylin stain. Oil *immersion*. *After* Zimmermann.

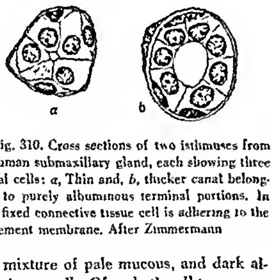

Fig. 310. Cross sections of two isthmuses from a human submaxillary gland, each showing three basal cells: *a*, Thin and, *b*, thicker canal belonging to purely albuminous terminal portions. In *b* a fixed connective tissue cell is adhering to the basement membrane. After Zimmermann

If the mucous cells are much more numerous the albuminous cells are pushed to the blind ends of the terminal portion or into saccular outpocketings. Here they form small groups which have the shape of thick-walled caps. In sections such caps appear as darkly staining crescents which surround the mucous tubules and are called *demilunes* of Gianuzzi. In the demilunes the albuminous cells are small, of irregular shape and often seem to be entirely separated from the lumen by the large mucous cells. However, there are always secretory capillaries which lead the secretion through the clefts between the mucous cells into the lumen (Fig. 308).

Basal (Basket) Cells. In all the glands of the oral cavity the epithelium in the terminal portion, as well as in the excretory ducts, is *provided with* peculiar basal or "*basket*" cells. In the secretory portion they lie between the glandular cells and the basement membrane; in cross section they appear as slender, curved spindles; usually only their nuclei can be discerned. When seen from the surface, in a tangential section of an albuminous secretory portion, they exhibit a stellate cell body with an angular, dark nucleus and many processes which contain coarse, straight, darkly staining fibrils. In the mucous tubules the basal cells are indistinctly outlined, flat structures with a nucleus and a parallel striation.

The basal cells, presumably of epithelial origin, are supposed to act as smooth muscle cells; through their contraction they facilitate the movement of the secretion into the excretory ducts; they are sometimes called "myo-epithelial cells." They are like the myo-epithelial cells of the sweat glands.

The Excretory Ducts. The necks or isthmuses are of variable length and are more or less branched. Their epithelium is of a low cuboidal type. Between its cells and the basement membrane, basal cells are scattered. The epithelium of the necks, as has been mentioned, often shows a mucoid transformation.

The epithelium of the striated tubules is a fairly regular, columnar epithelium; the lower parts of the cell bodies, between the nucleus and basement membrane, show a parallel striation, similar to that in the epithelium of the proximal convolutions of the kidney. This is generally believed to be caused by parallel rows of mitochondria (Fig. 308).

The epithelium of the striated tubules is believed by some to contribute water

cal demilunes are rare. In some individuals many of the albuminous cells show a slight mucoid reaction. The mucous cells are smaller than in the sublingual or in the pure mucous glands. Some of the isthmuses are short, others are long and branching. The striated tubules are numerous, very long, and have many branches.

portions abut directly on the striated tubules. The latter are very scarce and short and are sometimes represented by small groups of irregular, striated cells in the epithelium of the interlobular ducts.

In the *posterior lingual glands* the secretory portions are long-branching, sometimes anastomosing tubules. They contain only albuminous cells, which some-

Fig. 313 From a section of the sublingual gland of a nineteen-year old girl: *A*, *A'*, Interlobular ducts; *a*, artery, *ag*, small interlobular duct; *F*, extracted fat cells; *HM*, demilune; *SA*, serous intercalated duct; *SS*, mucous portion; *v*, vein 110 ×. After Schaffer

The *sublingual glands* are mixed glands with a markedly varying structure in their different parts. The mucous cells are far more numerous than in the mandibular gland, while the albuminous cells are in the minority and have a pronounced muco-albuminous character. For the most part they are arranged in thick demilunes and the isthmuses are extremely variable in length; many undergo a complete mucous transformation. so that the terminal

times show a slight reaction for mucus. These glands are rarely of mixed character. The system of the excretory ducts is poorly developed; isthmuses and short tubes are absent. These glands form a thin, serous secretion, which is found only on the furrows of the circumvallate papilla and evidently serves to wash out the taste buds.

The *glossopalatine glands* are pure mucous glands.

C. Glands of the tongue:

1. Anterior lingual gland (gland of Blandin or Nuhn) situated at the side of the median line under the apex of the tongue.
2. Posterior lingual glands.
   (a) Albuminous or gustatory glands (of v. Ebner) connected with the circumvallate papillae and opening into the circumvallate groove.
   (b) Mucous glands of the root of the tongue

D. Glands of the palate.

The following descriptions hold only for man:

The *parotid* is a pure albuminous gland. The secretory portions may have the form of grapes, but are usually elongated and branching. The necks are long and may branch several times. Their cells never undergo a mucous transformation. The striated tubules are fairly numerous. In the parotid gland of the newborn, however, the glandular cells often give a distinct staining reaction for mucus with mucicarmin and true mucous cells are often found at the transition of the secretory portion into the necks.

Fig. 312. From a section of the submaxillary gland of a fifty-year-old man: *F*, Fat cells from which the contents have been dissolved; *G*, blood vessel; *HM*, crescent; *IA*, interlobular duct passing over into a salivary duct; *IB*, interlobular connective tissue; *S*, intercalated duct passing over into a terminal portion; *SA*, serous alveolus, *Sp*, salivary duct, and cut tangentially, *Spt*; *SS*, mucous portion cut longitudinally and, *SS'*, cut across. *St*, short intercalated duct passing over into a mucous portion; *ST*, serous terminal portion. Mallory's connective tissue stain. 110 ×. After Schaffer.

In the *mandibular gland* of man the majority of the secretory portions are purely albuminous, while some are mucous with albuminous cells in the blind ends. Typi-

cal demilunes are rare. In some individuals many of the albuminous cells show a slight mucoid reaction. The mucous cells are smaller than in the sublingual or in the pure mucous glands. Some of the isthmuses are short, others are long and branching. The striated tubules are numerous, very long, and have many branches.

portions abut directly on the striated tubules. The latter are very scarce and short and are sometimes represented by small groups of irregular, striated cells in the epithelium of the interlobular ducts.

In the *posterior lingual glands* the secretory portions are long-branching, sometimes anastomosing tubules. They contain only albuminous cells, which some-

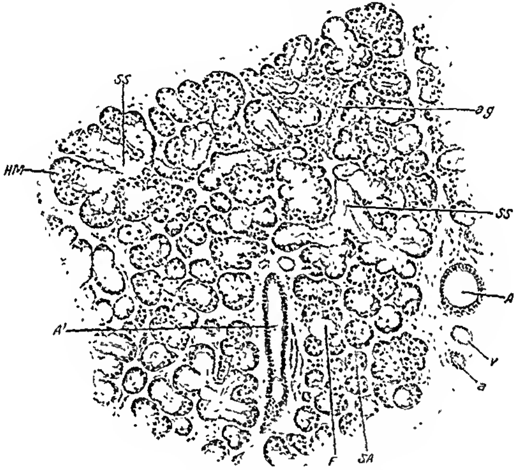

Fig 313. From a section of the sublingual gland of a nineteen-year-old girl. *A, A'*, Interlobular ducts; *a*, artery; *ag*, small interlobular duct; *F*, extracted fat cells; *HM*, demilune; *SA*, serous intercalated duct; *SS*, mucous portion; *v*, vein 110 ×. After Schaffer.

The *sublingual glands* are mixed glands with a markedly varying structure in their different parts. The mucous cells are far more numerous than in the mandibular gland, while the albuminous cells are in the minority and have a pronounced muco-albuminous character. For the most part they are arranged in thick demilunes and the isthmuses are extremely variable in length; many undergo a complete mucous transformation, so that the terminal

times show a slight reaction for mucus. These glands are rarely of mixed character. The system of the excretory ducts is poorly developed; isthmuses and short tubes are absent. These glands form a thin, serous secretion, which is found only on the furrows of the circumvallate papilla and evidently serves to wash out the taste buds.

The *glossopalatine glands* are pure mucous glands.

The *anterior lingual gland*, in its posterior part, consists of mixed branched tubules, which contain mucous cells, and are provided on their blind ends with very thin demilunes of muco-albuminous cells. The anterior part contains secretory portions with muco-albuminous cells only.

The *labial* and *buccal glands* are of the mixed type. The secretory portion sometimes contains only muco-albuminous cells, but in most cases the latter are confined to the blind end, while the rest of the cavity is lined with mucous cells. Some of the secretory parts may contain only mucous cells. As the necks are short and branch but little, the mucous secretory portions often pass directly into striated tubules.

The *glands of the root of the tongue* and the *palatine glands* are of the pure mucous variety. Short isthmuses have been found in the latter group.

In the various mammals the glands of the oral cavity show great structural variations. Even in closely related species the same gland may have a totally different cellular composition. In the dog and cat the mandibular gland consists for the most part of mucous cells, with a few albuminous cells forming typical demilunes. In the rodents, on the contrary, the same gland does not contain any mucin, and, therefore, is a pure albuminous gland. In the insectivora (hedgehog) the mandibular gland seems also to contain only albuminous cells, but these are of two different varieties. The term "sublingual gland" has been applied to different glands in various animals. The large gland, close to the mandibular, is now usually termed "retrolingual." In some animals (rodents) it has been described as a pure mucous, in others (dog, cat, pig) as a mixed gland with demilunes.

Interstitial Connective Tissue; Blood and Lymphatic Vessels. In the interstitial reticular connective tissue of the salivary glands are fibroblasts and macrophages, and fat cells scattered singly or in small groups; plasma cells are of common occurrence. Occasionally, small lymphocytes are also found. The larger blood vessels follow the excretory ducts; the loose capillary networks surround the ducts and the terminal portions. The lymph vessels are said to be scarce; they have not been studied adequately.

Nerves. Each salivary gland is provided with sensory nerve endings and two kinds of efferent secretory nerves, parasympathetic (cerebral) and sympathetic fibers. The cerebral preganglionic fibers for the mandibular and sublingual glands run in the chorda tympani nerve to the submaxillary ganglion; the sympathetic preganglionic fibers reach the superior cervical ganglion. From here the postganglionic fibers follow along the carotid artery. The vasodilators are believed to be included in the chorda tympani, the vasoconstrictors in the sympathetic nerves.

The parotid gland receives its secretory fibers from the glossopharyngeal nerve. In the interstitial tissue along the course of its blood vessels, *plexuses of myelinated (preganglionic and sensory)* and nonmyelinated fibers, and, close to the larger excretory ducts, groups of sympathetic multipolar nerve cells are found. On the outer surface of the terminal portions, nonmyelinated fibers form a network which sends small branches through the basement membranes. These branches form a second network on the inner surface of the membrane, and from this plexus small, final branches penetrate between the glandular cells, branch, and end on their surfaces with small, budlike thickenings.

Stimulation of the cerebral nerves of the mandibular gland causes the secretion of an abundant, thin saliva which is rich in water and salts, but poor in organic substances. Stimulation of the sympathetic nerve, on the contrary, yields a small quantity of thick saliva, with a high content of organic substances. The mechanism of the action of the nerves upon the glandular cells and the rôle of the vasodilators in the secretion are not known, and the existence of different kinds of nerve endings has not been proved. It is even doubtful whether the secretory fibers in the chorda tympani and in the sympathetic are of different nature.

After sectioning the chorda tympani nerve in the dog, the so-called "paralytic" secretion in the corresponding submaxillary and retrolingual glands occurs. This secretion is accompanied by intense degeneration and atrophy of the gland cells, especially the mucous elements in the retrolingual gland.

Histogenesis of the Glands of the Oral Cavity. Each gland arises at a certain time of fetal life, at a particular place in the wall of the embryonic oral cavity, through the growth of a solid epithelial bud into the subjacent mesenchyme. The large glands, as the submaxillary and the parotid, appear in embryos of six and eight weeks respectively; the smaller ones later. The epithelial bud grows and ramifies into a branched, treelike structure with club-shaped ends. It consists of undifferentiated polyhedral or cuboidal epithelial cells with many mitoses. Gradually a lumen appears in the older parts of the primordium and this canalization proceeds distally, but does not reach the terminal branches as long as these continue to grow and to form

additional buds. When the lumen reaches the terminal bud, the latter ceases to grow and only specific differentiation and enlargement of its cells occur. Mucigen appears in the mucous cells and zymogen granules in the serous ones. The histogenetic development of the glands continues after birth.

TONSILS

The aperture by which the oral cavity communicates with the next section of the digestive tract, the pharynx, is called the *fauces*. In this region the mucous membrane of the digestive tract contains accumulations of lymphatic tissue. Besides small, irregular infiltrations with lymphocytes, which may occur anywhere in this part of the mucous membrane, well outlined organs are formed by the lymphatic tissue. The surface epithelium invaginates them and they are called "tonsils." The lingual tonsils have been described (p. 357).

Between the glossopalatine and pharyngopalatine arches are the *palatine tonsils*. These are two oval, prominent accumulations of lymphatic tissue in the connective tissue of the mucous membrane, with ten to twenty deep *crypts*. In a cross section the stratified squamous epithelium of the free surface is seen to overlie a thin layer of fibrous connective tissue with papillae. The *crypts* almost reach the capsule on the opposite side and are of simple or irregularly branching form.

The nodules with their prominent centers are embedded in a diffuse mass of dense lymphatic tissue 1 to 2 mm. thick and are usually arranged in a single layer under the epithelium. The crypts with their surrounding sheaths of lymphatic

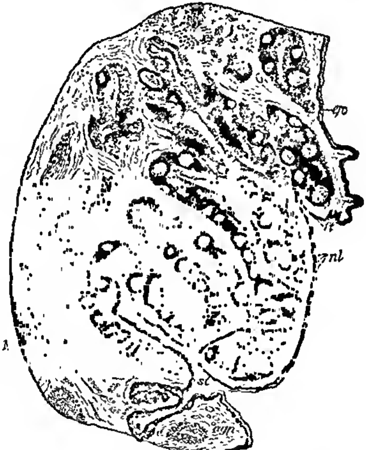

Fig. 314. Section through the palatine tonsil of man: *agp*. Glossopalatine arch; *ep*, stratified squamous epithelium; *ft*, crypt; *M*, striated muscle; *nl*, lymphoid nodules; *S*, connective tissue septa; *st*, remains of tonsillar sinus. 6½ ×. After Sobotta.

The *anterior lingual gland*, in its posterior part, consists of mixed branched tubules, which contain mucous cells, and are provided on their blind ends with very thin demilunes of muco-albuminous cells. The anterior part contains secretory portions with muco-albuminous cells only.

The *labial* and *buccal glands* are of the mixed type. The secretory portion sometimes contains only muco-albuminous cells, but in most cases the latter are confined to the blind end, while the rest of the cavity is lined with mucous cells. Some of the secretory parts may contain only mucous cells. As the necks are short and branch but little, the mucous secretory portions often pass directly into striated tubules.

The *glands of the root of the tongue* and the *palatine glands* are of the pure mucous variety. Short isthmuses have been found in the latter group.

In the various mammals the glands of the oral cavity show great structural variations. Even in closely related species the same gland may have a totally different cellular composition. In the dog and cat the mandibular gland consists for the most part of mucous cells, with a few albuminous cells forming typical demilunes. In the rodents, on the contrary, the same gland does not contain any mucin, and, therefore, is a pure albuminous gland. In the insectivora (hedgehog) the mandibular gland seems also to contain only albuminous cells, but these are of two different varieties. The term "sublingual gland" has been applied to different glands in various animals. The large gland, close to the mandibular, is now usually termed "retrolingual." In some animals (rodents) it has been described as a pure mucous, in others (dog, cat, pig) as a mixed gland with demilunes.

Interstitial Connective Tissue; Blood and Lymphatic Vessels. In the interstitial reticular connective tissue of the salivary glands are fibroblasts and macrophages, and fat cells scattered singly or in small groups; plasma cells are of common occurrence. Occasionally, small lymphocytes are also found The larger blood vessels follow the excretory ducts; the loose capillary networks surround the ducts and the terminal portions. The lymph vessels are said to be scarce; they have not been studied adequately.

Nerves. Each salivary gland is provided with sensory nerve endings and two kinds of efferent secretory nerves, parasympathetic (cerebral) and sympathetic fibers. The cerebral preganglionic fibers for the mandibular and sublingual glands run in the chorda tympani nerve to the submaxillary ganglion; the sympathetic preganglionic fibers reach the superior cervical ganglion. From here the postganglionic fibers follow along the carotid artery. The vasodilators are believed to be included in the chorda tympani, the vasoconstrictors in the sympathetic nerves.

The parotid gland receives its secretory fibers from the glossopharyngeal nerve. In the interstitial tissue along the course of its blood vessels, plexuses of myelinated (preganglionic and sensory) and nonmyelinated fibers, and, close to the larger excretory ducts, groups of sympathetic multipolar nerve cells are found. On the outer surface of the terminal portions, nonmyelinated fibers form a network which sends small branches through the basement membranes. These branches form a second network on the inner surface of the membrane, and from this plexus small, final branches penetrate between the glandular cells, branch, and end on their surfaces with small, budlike thickenings.

Stimulation of the cerebral nerves of the mandibular gland causes the secretion of an abundant, thin saliva which is rich in water and salts, but poor in organic substances. Stimulation of the sympathetic nerve, on the contrary, yields a small quantity of thick saliva, with a high content of organic substances. The mechanism of the action of the nerves upon the glandular cells and the rôle of the vasodilators in the secretion are not known, and the existence of different kinds of nerve endings has not been proved. It is even doubtful whether the secretory fibers in the chorda tympani and in the sympathetic are of different nature.

After sectioning the chorda tympani nerve in the dog, the so-called "paralytic" secretion in the corresponding submaxillary and retrolingual glands occurs. This secretion is accompanied by intense degeneration and atrophy of the gland cells, especially the mucous elements in the retrolingual gland.

Histogenesis of the Glands of the Oral Cavity. Each gland arises at a certain time of fetal life, at a particular place in the wall of the embryonic oral cavity, through the growth of a solid epithelial bud into the subjacent mesenchyme The large glands, as the submaxillary and the parotid, appear in embryos of six and eight weeks respectively; the smaller ones later. The epithelial bud grows and ramifies into a branched, treelike structure with club-shaped ends. It consists of undifferentiated polyhedral or cuboidal epithelial cells with many mitoses. Gradually a lumen appears in the older parts of the primordium and this canalization proceeds distally, but does not reach the terminal branches as long as these continue to grow and to form

movement. The salivary corpuscles which originate from heterophil leukocytes are recognized by the remnants of the granules and the polymorphous nucleus.

The lumen of the crypts may contain large accumulations of living and degenerated lymphocytes mixed with desquamated squamous epithelial cells, granular detritus and micro-organisms. These masses may increase in size and form large, cheesy plugs which are gradually eliminated. If they remain for a long time they may calcify. The micro-organisms are sometimes the cause of inflammation and suppuration; they may be responsible for some general infections.

Many small glands are connected with the palatine tonsils; their bodies are outside the capsule and their excretory ducts open for the most part on the free surface. Openings into the crypts seem to be very rare.

In the roof (fornix) and somewhat to the posterior wall of the nasal part of the pharynx is the unpaired *pharyngeal tonsil.* In this region the mucous membrane shows numerous folds, but no crypts. The epithelium on its surface is the same as in the rest of the respiratory passages—pseudostratified, ciliated columnar epithelium with many goblet cells. Small patches of stratified squamous epithelium are very common, however. The epithelium is abundantly infiltrated with lymphocytes, especially on the crests of the folds. A 2 mm. thick layer of diffuse and nodular lymphatic tissue is found under the epithelium and participates in the formation of its folds; it is separated from the surrounding parts by a thin capsule which contains many elastic networks and sends thin partitions into the core of the folds. Outside the capsule there are small glands of mixed character. Their ducts—often markedly dilated—traverse the lymphatic tissue and empty into the furrows or on the free surface of the folds.

Other small accumulations of lymphatic tissue have been described in the mucous membrane of the pharynx, around the orifices of the Eustachian tube behind the pharyngopalatine arches, and in small granules in the posterior wall.

Unlike the lymph nodes, the tonsils do not possess lymphatic sinuses and lymph is not filtered through them. They are provided, however, with netlike plexuses of wide, blindly ending lymph capillaries which surround their outer surface.

The tonsils generally reach their maximal development in childhood. The involution of the palatine tonsils seems to begin about the age of fifteen, while the follicles of the root of the tongue persist longer. The pharyngeal tonsil in the adult is usually found in an atrophic condition with its ciliated epithelium in great part replaced by stratified squamous epithelium.

The participation of the tonsils in the new formation of lymphocytes is the only established function that can be ascribed to them. A characteristic feature of the tonsil is the infiltration of its epithelium by lymphocytes. This infiltration is manifest along the entire digestive tract; it occurs diffusely in the small intestine, and is concentrated in the tonsils, in the pharynx and the solitary and aggregated nodules in the intestine.

It is generally believed but not proved that the infiltration with lymphocytes has something to do with the protection of the organism against the penetration of various noxious agents and especially of micro-organisms into the body. Pathogenic bacteria have been found in the lymphatic tissue of the tonsils and the nodules of the intestine as an apparently normal phenomenon. It has been suggested that these bacteria penetrating into the lymphatic tissue are modified and made less virulent, and that they then act as antigens and instigate the production of antibodies. On the other hand, the tonsils and the nodules of the intestine have been shown to be the portals of entry for pathogenic micro-organisms and general infections have been traced from them.

The palatine tonsils develop from the rudiments of the dorsal part of the second gill pouch. During the fourth month of fetal life the epithelium pushes solid outgrowths into the subjacent connective tissue; these later become hollow. Around

tissue are partially separated from one another by thin partitions of loose connective tissue which invaginate from the capsule. In this connective tissue there are always lymphocytes of various sizes, and mast and plasma cells which are often degenerating. The presence of large numbers of heterophil leukocytes indicates inflammation, which is very common in mild degree. Frequently there are islands of cartilage or bone which probably indicate a pathologic process. In the deeper portions of the crypts, the limit between the epithelium and the lymphatic tissue is effaced in most places by an intense infiltration of the epithelium with lymphocytes. The epithelial cells are pushed aside and disfigured so that sometimes only a few of them remain on the surface. Heterophil leukocytes are always present in small numbers. Plasma cells are very common here.

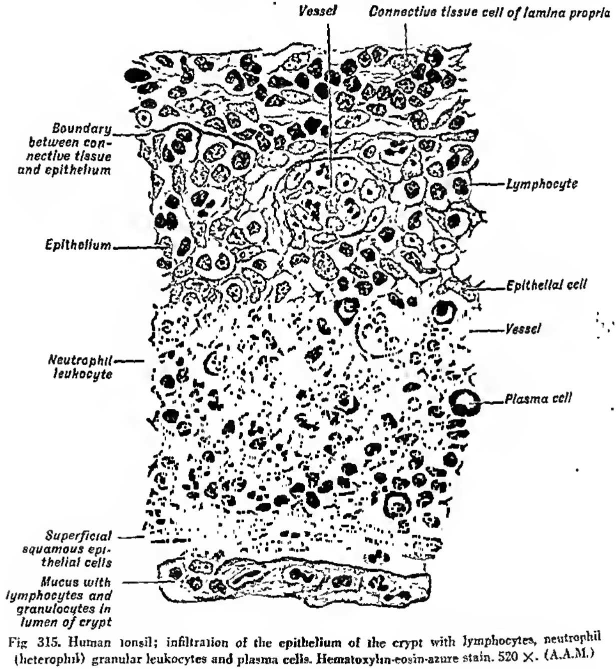

Fig 315. Human tonsil; infiltration of the epithelium of the crypt with lymphocytes, neutrophil (heterophil) granular leukocytes and plasma cells. Hematoxylin-eosin-azure stain. 520 ×. (A.A.M.)

The lymphocytes which pass through the epithelium are found in the saliva as the *salivary corpuscles*. They appear here usually as degenerating, vesicular elements with a more or less constricted nucleus and granules which show Brownian

these epithelial growths, lymphatic tissue gradually develops through isolation and mobilization of mesenchyme cells which are transformed into lymphocytes, while the cells which remain fixed furnish the reticular framework.

THE PHARYNX

The posterior continuation of the oral cavity is the pharynx. In this section of the digestive tract the respiratory passages and the pathway for the food cross and fuse with each other. The upper part of the pharynx is the nasal, the middle the oral, and the lower the laryngeal portion. In the upper part it approaches the structure of the respiratory system, while in the lower it corresponds more to the general plan of the digestive tube.

Instead of a muscularis mucosae, the mucous membrane is provided with a thick and dense, netlike, mainly longitudinal, elastic layer. A loose submucous layer is well developed only in the lateral sides of the nasal part of the pharynx and where the pharynx continues into the esophagus; here the elastic layer soon becomes thinner. In all other places the mucous membrane is directly adjacent to the muscular wall which consists of an inner longitudinal and an outer oblique or circular layer of striated muscle. The elastic layer fuses with the interstitial tissue of the muscle and sends strands of elastic fibers between the muscular bundles. In the fornix it is fused with the periosteum of the base of the skull.

The lamina propria mucosae consists of dense connective tissue containing fine elastic networks; those places covered with stratified squamous epithelium are provided with small papillae. In the area covered with pseudostratified ciliated columnar epithelium there are no papillae; a distinct basement membrane separates the epithelium from the connective tissue.

The two lower sections of the pharynx and a part of the nasal region have stratified squamous epithelium; toward the roof (fornix) of the pharynx its epithelium becomes first stratified columnar and then pseudostratified columnar ciliated, with many goblet cells. On the lateral sides of the nasal part this ciliated epithelium continues downward beyond the aperture of the Eustachian tube. With age the ciliated epithelium may be replaced by stratified squamous epithelium over large areas.

The pharynx possesses two kinds of small glands (Fig. 317). Glands of a pure mucous type are found in those places lined with stratified squamous epithelium. They are always located under the elastic layer, sometimes deep in the muscle. Glands of mixed type, similar to those of the dorsal surface of the soft palate, are confined to the regions covered with ciliated epithelium.

The arrangement of the blood vessels in the pharyngeal mucous membrane is similar to that of the oral cavity. Lymphatic capillaries are abundant and form dense networks in the mucous membrane in the neighborhood of the tonsils.

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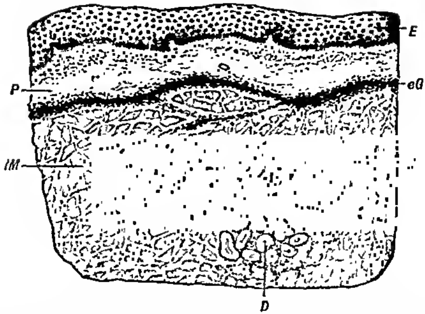

Fig. 316. Cross section through the posterior pharyngeal wall of a human adult: *E*, Stratified squamous epithelium; *P*, lamina propria mucosae; *eG*, elastic boundary layer; *IM*, longitudinal muscular layer; *D*, mucous glands which penetrate deep into the muscle. Resorcin-fuchsin and hematoxylin stains. 50 ×. After Schumacher.

Opening of a
umu-
ished
aside by the body of the gland. 27 ×. After Schaffer.

from the connective tissue. The most primitive type of teeth, in which the character of cutaneous papillae is quite evident, is found in the placoid scales scattered all over the surface of the body of the selachians. Similar structures develop in many parallel rows in the mucous membrane *of the oral cavity of the fishes*, where they are subject to continuous renewal during life.

Two sets of teeth have to be distinguished in man and most mammals. The first set forms the *deciduous teeth* of childhood; their eruption starts about the seventh month after birth and they are shed between the sixth and thirteenth years. They are gradually replaced by the *permanent teeth*. The microscopic structure of both kinds of teeth is similar in principle, but the permanent tooth reaches a higher development. Each of the various types of teeth in each set has a different form adapted to its specific functions, *i. e.*, the incisors for biting and the molars for grinding and pounding the food.

All teeth consist of the same two portions, the *crown*, which projects above the gum, and the *root* which, tapering down to its tip, fits closely into an excavation of the maxillary or mandibular bone, the *alveolus*. The place where the crown and the root meet is sometimes called the *neck*. The lower molars have two, the upper molars three, roots. The tooth contains a small cavity which corresponds roughly with the outer form of the tooth. It is called the *pulp cavity* and continues into each root as a narrow canal that communicates through one or more openings at the apex of the root with the *periodontal membrane*.

The *hard portions of a tooth* consist of three different tissues: Dentin, enamel and cementum. The bulk of the tooth is formed by the *dentin* or the ivory which everywhere surrounds the pulp cavity. It is thickest in the crown and tapers down to the points of the roots. Its outer surface is covered, in the region of the crown, by a layer of *enamel*, which reaches its greatest thickness on the exposed part of the crown and thins down toward the neck. In the region of the root the dentin is covered by a thin layer of *cementum*

Fig. 319. Longitudinal ground section of a human cuspid. The top of the crown has been abraded: *a*, Parallel stripes of Retzius; *b*, *Schreger's lines of the enamel*; *c*, large interglobular space (of Owen); *d*, dentine; *e*, Tomes' granular layer of the dentin; *f*, cell-free and, *g*, cellular cementum of root; *p*, pulp cavity. 7 ×. After v. Ebner, from Schaffer.

Chapter XVII

THE TEETH

The teeth are derivatives of the oral mucous membrane. They may be considered as modified papillae whose surface is covered by a thick layer of a peculiar, hard tissue. A part of their hard substance originates from the epithelium, the rest

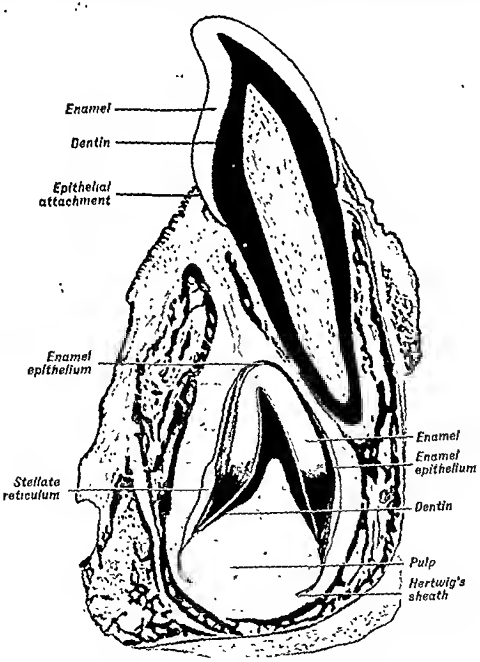

Fig. 318. Diagram of a deciduous tooth and its corresponding permanent tooth germ (below). Note the surrounding alveolar bone. Retouched photomicrograph. 8 ×. Courtesy of B. Orban.

toward the periphery and penetrate every part of the dentin. At their origin in the innermost part of the dentin, their diameter is 1.3 to 2.2 μ; in the outer portions they become narrower. On their way from the pulp cavity most of the tubules describe an S-shaped curve. The tubules branch and, especially in the outer layers of dentin, frequently form loop-shaped anastomoses.

The layer of dentin which is immediately adjacent to the tubules and surrounds these as a sheath of Neumann differs, by its high refringence and distinct staining in decalcified specimens, from the rest of the dentin.

Between the dentinal tubules are systems of collagenous fibrils arranged in thin bundles 2 to 4 μ thick and kept together by a cementing substance; they correspond with the ossein fibrils of bone.

The course of the fibrillar bundles is said to be, in general, parallel to the long axis of the tooth and perpendicular to the dentinal tubules. They also run obliquely and around the tubules; in the crown they are tangential to the free surface. The fibrils in the adjacent layers form angles of varying degrees—smaller in the outermost portions of the dentin and larger in the proximity of the pulp cavity.

Some investigators distinguish a peripheral layer of *cover dentin,* with coarser fibers, from the *circumpulpar dentin,* which forms the inner mass and consists of thinner fibrils.

The calcification of the developing dentin is not always complete and uniform. The deposits of calcium salts which appear during development in the organic ground substance, have the form of spheres which gradually gain in size and finally fuse. In incompletely calcified regions, between the calcified spheres, there remain angular "interglobular" spaces which contain only the organic matrix of the dentin. The dentinal tubules continue without interruption through the spheres and through the interglobular spaces. In a macerated tooth, from which all organic parts have disappeared, the tubules as well as the interglobular spaces are filled with air and appear dark in transmitted light. In many otherwise normal teeth there are layers of large, interglobular spaces in the deeper parts of the enamel-covered dentin of the crown—the *lines of Owen.* In the region of the root there is always a layer of very small interglobular spaces immediately under its dentino-cemental junction. Under low magnification this layer has a granular appearance; it is called the *granular layer of Tomes* (Fig. 319, *e*).

In sections through a decalcified tooth fixed with its soft parts, each dentinal tubule is seen to contain a protoplasmic fiber (of Tomes) which in life probably completely fills the lumen of the tubule, but which in fixed preparations appears considerably shrunken. When the tubules are seen in cross section, each small oval contains a dark dot. These fibers of Tomes are processes of the *odontoblasts,* which are arranged on the wall of the pulp cavity and send their protoplasmic processes into the dentinal tubules.

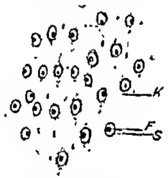

Fig. 321. Tangential section through the root of a molar of an ape: *F,* Shrunken dentinal fiber; *S,* margin of the tubules (Neumann's sheath); *K,* matrix. 740 ×. After Schaffer.

The dentin is sensitive to touch, to cold, to acid-containing foods, etc. Only occasional nerve fibers penetrate the dentin and these extend for but short distances. It is believed that the fibers of Tomes transmit the sensory stimulation to the pulp which contains many nerves.

With the aid of radioactive phosphorus it has been shown that there is an active interchange of calcium and phosphorus between dentin and enamel on one hand and the blood on the other. The interchange persists on a diminished scale

which leaves the opening of the canal free. The edge of the enamel meets the cementum at the neck.

The *soft parts* associated with the tooth are: (1) The pulp, which fills the pulp cavity; (2) the periodontal membrane which connects the cementum-covered surface of the root with the bone of the alveolus; (3) the gum, which is that portion of the oral mucous membrane surrounding the tooth. In young individuals the gum is attached to the enamel; with increasing age the gum gradually recedes from the enamel, so that in old people it is attached to the cementum.

Dentin. The dentin is of yellowish color and semitransparent in fresh condition; when dried after maceration it acquires a silky appearance because air has entered its tubules. It is harder than compact bone, although it resembles bone in its structure, chemical nature, and development.

As in bone, the substance of the macerated dentin consists of an organic (28 per cent) and an inorganic (72 per cent) part. They can be separated from each other either by decalcification in mineral acids, when the organic part remains and the substance becomes soft, or by incineration when only the inorganic material remains. The latter is much the same as in bone except that it is denser and less soluble (see p. 128). The organic part dissolves in boiling water and yields a solution of gelatin.

In a ground section passing through the axis of a macerated tooth, the dentin has a radially striated appearance. This is caused by the presence in it of innumerable, very fine canals, the *dentinal tubules*, which diverge from the pulp cavity

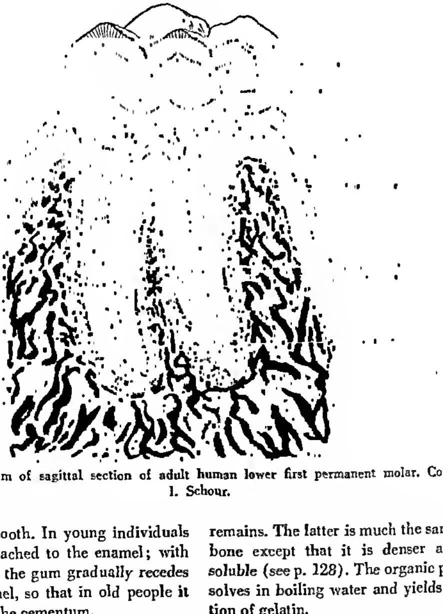

Fig. 320. Diagram of sagittal section of adult human lower first permanent molar. Courtesy of I. Schour.

face the dentin and their cross sections show a scalelike formation (Fig. 323).

This form and arrangement is explained by calcification beginning earlier on the side of the rods which lies nearest to the dentin. This inner, harder side is supposed to press into the softer side of the adjacent rod, compressing it and leaving one or two groovelike impressions.

The exact course of the enamel rods is extremely complicated and seems to be perfectly adapted to the mechanical requirements connected with the grinding and pounding of food. Starting from the dentin the rods run perpendicularly to the surface; in the middle zone of the enamel they bend spirally and in the outer zone again assume a direction perpendicular to the surface. In addition, the rods show numerous, small, wavy curves. On the lateral surfaces of the crown, the rods are arranged in zones which encircle the tooth in horizontal planes. The bends of the rods in two neighboring zones cross one another. In axial, longitudinal, ground sections, the crossing of groups of rods appears in reflected light as light and dark lines, more or less perpendicular to the surface—the lines of Schreger (Fig. 319, *b*).

In a cross section of the crown the enamel shows concentric lines which are brown in transmitted light and colorless in reflected light. In longitudinal, axial sections they are seen to run obliquely inward from the surface and toward the root. They are called the *lines of Retzius and are connected* with the circular striation on the surface of the crown.

The free surface of the enamel is covered by two *membranes*. The inner is about 1 μ thick and is the last product of the activity of the ganoblasts before they disappear (see below). It is somewhat more resistant to acid than the rest of the enamel as it contains less calcium. A second membrane, external to the first, is related to keratin. It is 2 to 10 μ thick and resistant to acids as well as alkalis. In the adult both membranes are gradually worn off.

In an axial section of the tooth the line of *junction between the dentin* and the enamel (*dentino-enamel junction*) is uneven and scalloped. Pointed processes of dentin penetrate the enamel and are separated from one another by excavations. Some dentinal tubules penetrate the enamel and end blindly. The spindle-shaped processes of the dentinal matrix penetrating a short distance into the enamel are called *enamel spindles.*

Local disturbances of the enamel during development cause the so-called *enamel lamellae* and *tufts.* These lamellae are organic material extending from the surface of the enamel toward and sometimes into the dentin. The tufts extend from the dentino-enamel junction into the enamel for one third of its thickness. The tuftlike shape, however, is an optical illusion due to the projection of fibers lying in different planes into one plane. They are groups of poorly calcified, twisted rods with abundant cementing substance between them.

Cementum. The cementum covering most of the root is coarsely fibrillated, interstitial bone substance. Near the apex bone cells are embedded in it. Canaliculi, Haversian systems and blood vessels are normally absent. The layer of cementum increases in thickness with age, especially near the end of the root, and then Haversian systems with blood vessels may appear. Coarse collagenous bundles from the periodontal membrane penetrate the cementum. These fibers of Sharpey (p. 132) remain uncalcified and in ground sections of the macerated tooth appear as empty canals.

Unlike the high resistance of the dentin, which may remain unchanged even after the destruction of the pulp and odontoblasts and after the "filling" of the pulp cavity, the cementum readily undergoes necrosis when the periodontal membrane is destroyed and may be resorbed by the surrounding connective tissue; on the other hand, new layers of cementum may be deposited on the surface of the root. If this deposition becomes extensive it may

via the dentino-cemental junction in teeth in which the *pulp cavity* has been filled.

The difference between dentin and bone consists of the position of the cells in relation to the intercellular substance. While in bone the cells are evenly distributed in the hard intercellular substance and send their processes out in all directions, the cells of the dentin remain on the surface of the intercellular substance and only send their processes into it. Although the odontoblasts undoubtedly play a rôle in the nutrition of the dentin, the latter does not become necrotic after the destruction of the pulp and the "filling" of a tooth.

Fig. 322 Ground cross section of the crown of a human cuspid. *D*, dentin, *G*, wavy boundary between enamel and dentin. *O*, the surface of the tooth; *RL*, parallel stripes of Retzius; *SB*, enamel tuft 80 ×. After Schaffer

In old age the dentinal tubules are often obliterated through calcification; the dentin then becomes transparent When the dentin is denuded because of extensive abrasion of the crown, or when the outside of the tooth is irritated, a production of new or "secondary" dentin of irregular structure may often be observed on the wall of the pulp cavity. This may be so extensive as to fill the cavity completely.

Enamel. The enamel, a cuticular formation of epithelial origin, is the hardest substance found in the body; it gives sparks with steel. It is bluish-white and transparent in thin ground sections. When fully developed, enamel consists almost entirely of calcium salts in the form of large apatite crystals (p. 126), while only 3 to 5 per cent of it is organic substance. Consequently, after decalcification of a fully developed tooth, the enamel is, as a rule, completely dissolved.

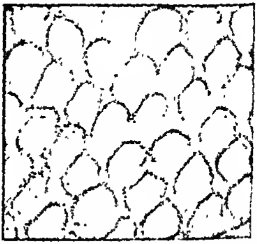

Fig. 323. Cross section of human enamel rods. The dark lines are the cementing substance between the pale rods. Photomicrograph. High magnification. Courtesy of B. Orban.

The enamel consists of thin prisms or rods which stand upright on the surface of the dentin, usually with a pronounced inclination toward the crown. They are kept together by a small amount of cement substance. Every rod runs through the whole thickness of the enamel layer. This, however, cannot be seen in sections of the enamel because the rods are twisted.

The substance of a rod in its longitudinal section seems homogeneous in a ground preparation. But after acid acts upon such a section, a distinct cross striation appears in the rods; this indicates that the calcification probably proceeds by layers.

In the human tooth most of the rods in cross section have the form of fluted semicircles. The convex surfaces of all rods

generate and undergo calcification, giving rise to the *cementicles.*

The Gum. The gum is that part of the mucous membrane which is firmly connected with the periosteum at the crest of the alveolar bone. It is also linked to the surface of the tooth by the *epithelial attachment of Gottlieb,* which gradually approaches the apex of the tooth with advancing age. The gum has very high papillae. The epithelial attachment is devoid of papillae except when chronically inflamed (see Fig. 318). Between the epithelium and the enamel there is a small furrow surrounding the crown, the gingival crevice. No glands are found in the gums.

Histogenesis of the Teeth. The enamel is a product of the ectodermal epithelium; all the other parts are derivatives of the connective tissue.

In human embryos of the fifth week the ectodermal epithelium lining the oral cavity presents a thickening along the edge of the future upper and lower jaws. The thickening consists of two solid epithelial ridges which extend into the subjacent mesenchyme. Of these, the labial ridge later splits and forms the space between lip and alveolar process of the jaw. The lingual ridge, nearer the tongue, produces teeth and is called the *dental lamina.* According to most recent investigators both ridges are independent from the beginning.

The edge of the dental lamina extends into the connective tissue of the jaw and shows at several points budlike thickenings—the primordia of the teeth, the *tooth germs.*

There are ten tooth germs in each jaw, one for each deciduous tooth. In each germ a dense group of epithelial cells becomes conspicuous as the *enamel knot;* it is a temporary structure and later disappears. The cells of the mesenchyme under the enamel knot form a dense group, the primordium of the papilla.

The dental lamina then extends beyond the last deciduous tooth germ and slowly forms germs of the permanent molars, which are not preceded by corresponding deciduous teeth.

Beginning with the tenth to twelfth week the remainder of the dental lamina again produces solid epithelial buds—the *germs for the permanent teeth*—one on the lingual side of each deciduous germ. After the formation of the permanent tooth germs the dental lamina disappears. The transformations of the permanent tooth germ are *the same as in the deciduous germ.*

The papilla enlarges and invaginates the base of the epithelial tooth germ (Fig. 327, *ZP*). The latter, while still connected by an epithelial strand with the dental lamina, becomes bell-shaped and caps the convex surface of the papilla. From now on it is called the *enamel organ,* because it produces the enamel in its further development. Both the papilla and the enamel organ gradually gain in height and the latter soon acquires approximately the shape of the future organ.

Fig. 325. Portion of a ground cross section through the lower part of a root of a macerated human tooth. Air has filled the lacunae: *KL,* Refractile boundary between the apparently lamellated layers; *SF,* uncalcified Sharpey's fibers; *TK,* Tomes' granular layer; *Z,* dentin; *ZK,* cementum corpuscles. 80 ×. After Schaffer.

A concentric layer of connective tissue, the dental sac, develops around the tooth primordium and interrupts its epithelial connection with the oral cavity. Around the sac and at a certain distance from it the bone of the jaw develops.

The peripheral cells of the enamel organ are arranged in a regular, radial fashion. On the convex surface the outer enamel epithelium remains small and cuboidal. On the invaginated base the cells of the inner enamel epithelium become tall and regular. They help in the elaboration of the enamel and are called *ameloblasts* or *ganoblasts.* Their attachments are provided with a system of terminal bars. In the inner mass of epithelial cells a clear liquid accumulates between the cell bodies which remain connected with one another by long processes. The epithelium thus acquires a reticular connective tissue-

be called *cementum hyperplasia* and is a favorable reaction to irritation.

Pulp. The pulp of the tooth fills the pulp cavity and is the connective tissue which formed the dental papillae during embryonic development. In the adult tooth it has an abundant, gelatinous, basophil ground substance similar to that of mucoid tissue. It contains a multitude of thin collagenous fibrils running in all directions and not combined into bundles.

Fig. 324. Dentino-enamel junction of a tooth of a man. Ground section. The enamel prisms appear as a fine, wavy striation. The interglobular spaces in the dentin are black (air filled). Between these lacunae are the dentinal tubules 80 ×. After Braus.

The spindle- or star-shaped cells suggest embryonic mesenchymal elements; macrophages and ameboid wandering cells are also present. The cells of the pulp adjacent to the dentin are large, elongated, and radially arranged in the fashion of an epithelium; they are called *odontoblasts* and contain mitochondria and a Golgi net in their central part. The odontoblasts send one or more processes into the dentinal tubules; these are the fibers of Tomes described above.

The pulp continues into the narrow canal of the root where it surrounds the blood vessels and nerves, and continues through the openings in the apex into the periodontal membrane. The pulp contains many blood vessels. Several small arteries enter each root and are accompanied by veins. The arteries give rise to a dense network of wide capillaries whose loops reach the layer of the odontoblasts and then continue into the veins which occupy a more central position. True lymphatic capillaries have been found recently by some investigators. Numerous bundles of myelinated nerve fibers, which arise from small cells in the gasserian ganglion, enter the pulp cavity through the canals of the root. They form a plexus in the pulp from which a finer plexus of nonmyelinated fibers in the peripheral layers arises; nerve endings have been described between the odontoblasts.

Periodontal Membrane. The periodontal membrane, which also serves as periosteum to the alveolar bone, furnishes a firm connection between the root and the bone. It differs from the usual periosteum by the absence of elastic fibers. It consists of thick collagenous bundles, which generally run obliquely from the alveolar wall to the cementum. At the bottom of the alveolar cavity they are thinner, and the softer tissue continues into the pulp. At the neck of the tooth the fibers are especially prominent, are firmly attached to the cementum and are called the "horizontal groups of fibers" of the tooth. Nearer the surface they run from the bone upward to the edge of the cementum. The fiber bundles of the periodontal membrane have a slightly wavy course; when the tooth is not functioning they are relaxed and permit a slight movement of the tooth upon the application of stress.

In many places in the periodontal membrane, blood and lymph vessels and nerves embedded in a small amount of loose connective tissue, and small islands of epithelium are scattered, especially near the surface of the cementum. These islands are vestiges of the epithelial sheath of Hertwig. The epithelial rests frequently de-

When the dentin first appears it is a soft fibrillar substance—the *predentin*. The fibrils are continuations of the fibrils of the papilla. They are of the argyrophil type and are generally called *Korff's fibers*. They enter the dentin, spread out fanlike, and change into the collagenous, fibrillated matrix of the dentin (Fig. 330).

In dentin formation, calcification follows closely the deposition of the fibrillar soft substance. But during the whole process there is always a thin layer of uncalcified dentin near the odontoblasts.

The process of dentin formation is much the same in nature as the formation of bone. Almost immediately after the appearance of the first calcified dentin on the convexity of the papilla, the ganoblasts begin the elaboration of enamel. It is deposited layer by layer on the surface of the calcifying dentin.

The ganoblasts grow into tall and regular, columnar cells; in the earlier stages each cell contains a cytocentrum and a Golgi net above the elongated, oval nucleus. The attached part of the cell contains granular material which stains brown with osmic acid. In vitally stained animals this part stores the dyes in granular form. On the slopes of the papilla the ganoblasts become lower and at the base of the papilla they continue into the outer enamel epithelium.

As the mass of enamel increases, the ganoblasts recede and their basal surfaces remain covered by thin, cuticular plates and connected with one another by terminal bars. The most recent investigations demonstrate the development of the enamel rods from their beginning as individual rods and not from a homogeneous mass. Thus the *Tomes' processes* are the primordia of the enamel rods; each corresponds to a separate ganoblast and remains connected with it until the enamel is complete. It is probable that the processes of Tomes are a cuticular secretion of the basal ends of the ganoblasts.

Calcification starts at the periphery of each row and proceeds toward its interio cementing substance finally calc organic material remains in the e

Fig. 327. Primordium of the right lower central incisor of a human embryo of ninety-one days, in sagittal section. Collagenous fibers black: *ASE*, External enamel epithelium; *EW*, epithelium of the dental lamina; *H*, neck of the enamel organ; *LE*, labial epithelium; *ME*, epithelium on the floor of the mouth; *Mp*, preformed membrane; *SE*, internal enamel epithelium; *SP*, enamel pulp; *SSt*, enamel cord; *ZL*, internal end of the dental lamina; *ZP*, dental papilla; *ZS*, dental follicle. Mallory's connective tissue stain. 80 ×. After Schaffer.

like appearance—the *stellate reticulum* (enamel pulp).

When the formation of the hard tooth substances begins (embryos of about twenty weeks) the mesenchyme of the papilla contains numerous blood vessels and a few reticular fibrils between its cells. The cells adjacent to the layer of ganoblasts become transformed into odontoblasts (Fig. 328, *O*).

The dentin first appears as a thick limiting line between ganoblasts and odontoblasts, sometimes called the *membrana preformata*. Some believe that the odontoblasts do not form the dentin, but are probably concerned in its nourishment and possibly with the deposition of calcium in it.

The layer of dentin extends down the slopes of the papillae. It gradually grows thicker and is transformed into a solid cap of dentin through the apposition of new layers on its concave surface. As the odontoblasts recede from the dentin, thin processes of their cytoplasm remain in the mass of deposited dentin as the dentinal fibers.

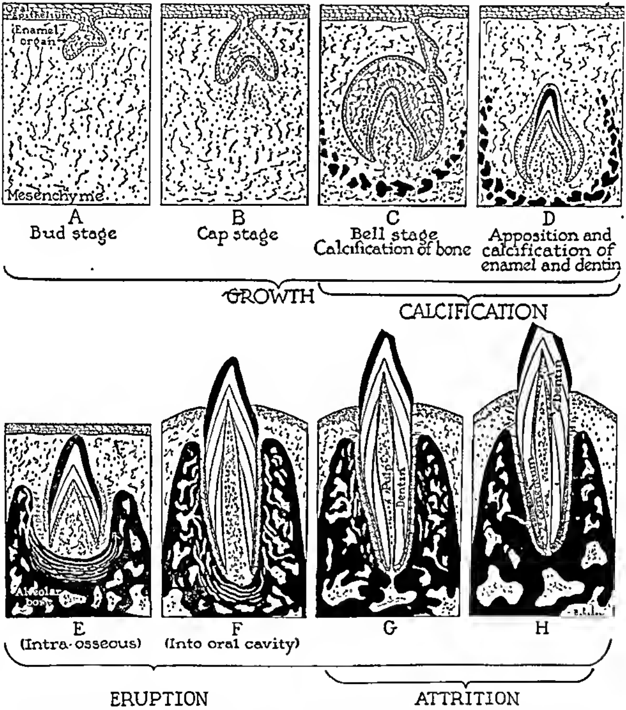

Fig. 326. Diagram of life cycle of a human deciduous incisor. The normal resorption of the root is not indicated. Enamel and bone are drawn in black. Slightly modified after Schour and Massler.

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Calcification starts at the periphery of each row and proceeds toward its interior. When the cementing substance finally calcifies, so little organic material remains in the enamel that it

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is completely dissolved in decalcification. Complete calcification is not reached until very late and for a long time dyes and other substances may penetrate the partly calcified enamel. That the calcification is seldom absolutely uniform has been mentioned above. One of the most striking causes of hypocalcification is parathyroidectomy.

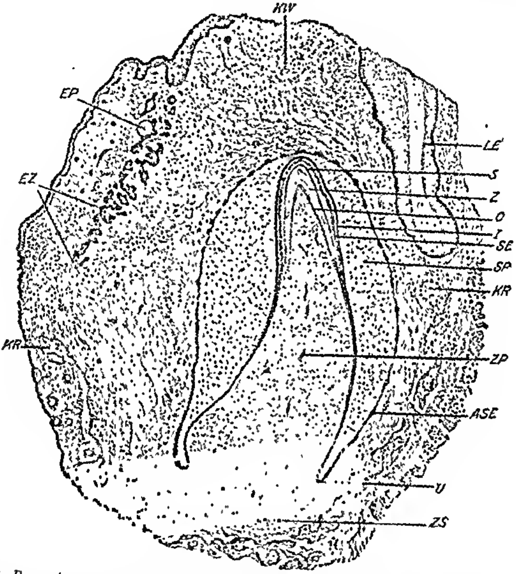

Fig. 328. Primordium of the lower central incisor of a five months' embryo. Sagittal section: *ASE*, External enamel epithelium, *EP*, epithelial pearls in the rests of the permanent dental lamina, *EZ*; *I*, stratum intermedium; *KR*, alveolar bone; *KW*, gum wall; *LE*, stratified squamous epithelium of the lip; *O*, primordium of the odontoblasts; *S*, enamel cap; *SE*, internal enamel epithelium; *SP*, enamel pulp; *U*, transition of the external into the internal enamel epithelium; *Z*, dentin; *ZP*, dental pulp; *ZS*, dental follicle 30 × After Schaffer.

Schour (1936) studied the rate of deposition of enamel with sodium fluoride and of dentin with vital injections of alizarine He found that the daily thickening of dentin is about 4 μ and that unusual increments (*neonatal lines*) appear in the enamel and dentin formed in the deciduous teeth at the time of birth.

The disturbances in the *development* of the teeth due to vitamin deficiencies are described by M. Mellanby (1928).

When the definitive thickness and extension of the enamel capsule is reached in the neck region, the ganoblasts become small cuboidal cells and then atrophy. Before they disappear, they elaborate the inner cuticle of the enamel which covers the ends of the rods.

At the end of the enamel organ, the outer and inner enamel epithelium form a fold, the *epithelial sheath of Hertwig*. The *development of the root* begins shortly before the eruption of the tooth, continues after the crown has emerged from within the mucous membrane, and is not completed until much later; the epithelial sheath disappears when the root development is finished.

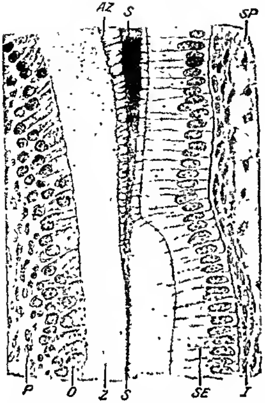

Fig. 329. Formation of enamel and dentin: *AZ*, Surface of the dentin eroded through the first enamel layer; *I*, intermediate layer of the enamel organ; *O*, layer of odontoblasts; *P*, tooth papilla; *S*, first layer of enamel from which shrinkage has partially raised the enamel epithelium, *SE*; *SP*, enamel pulp; *Z*, dentin. 380 ×. After Schaffer.

When the germ of the permanent tooth begins to develop, its growth pressure causes resorption first of the bony partition between the two teeth, then of the root, and eventually even of a part of the enamel of the deciduous tooth. Osteoclasts are prominent in this process of destruction just as in the resorption of bone. The crown of the permanent tooth moving upward gradually takes the place of the former deciduous crown.

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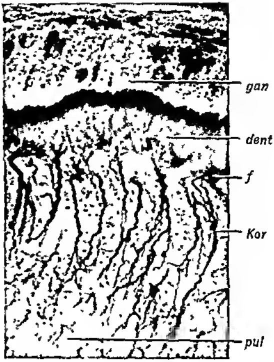

Fig. 330. The continuation of Korff's fibers of the pulp into the matrix of the dentin: *gan*, Ganoblasts; *dent*, dentin; *f*, spreading of Korff's fibers (*Kor*) at the dentin border; *pul*, tooth pulp. Photomicrograph. 700 ×. Courtesy of B. Orban.

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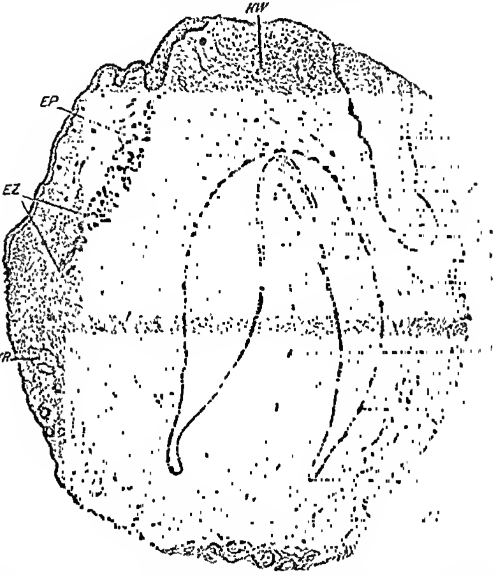

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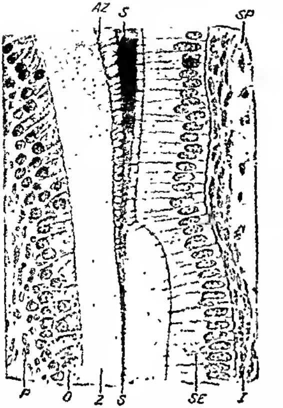

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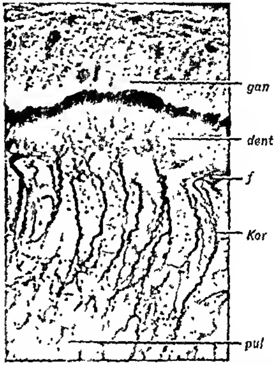

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ESOPHAGUS AND STOMACH

THE ESOPHAGUS

THE esophagus is a muscular tube which conveys the food rapidly from the pharynx to the stomach. Its wall presents all

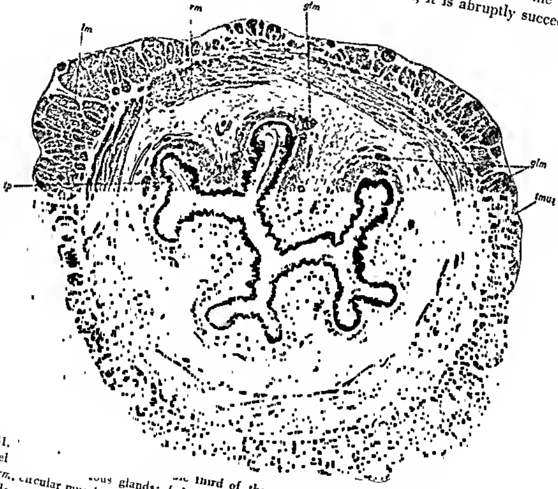

Fig 331. ... third of the esophagus of a twenty-eight-year-old man. ep, Epithel... ...ous glands; l, lumen; lm, longitudinal muscle; mm, lamina muscularis mucosae; rm, circular muscle; su, submucosa; tmus, tunica muscularis; tp, lamina propria mucosae; i, inner longitudinal muscle 8 × After Sobotta.

the layers enumerated on p. 350 for the digestive tube in general.

The mucous membrane is 500 to 800 μ thick. The epithelium is of the stratified squamous variety. It continues into the esophagus from the pharynx. At the transition of the esophagus into the stomach in the cardia, it is abruptly succeeded by the simple columnar epithelium of the stomach (Fig. 335). On macroscopical examination the boundary line between the smooth white mucous membrane of the esophagus and the pink surface of the

gastric mucosa appears as a jagged line.

In man the flattened cells of the superficial layers of the epithelium contain a small number of keratohyalin granules, but do not undergo true cornification. The lamina propria is bent by numerous longitudinal ridges which fuse with one another in many places and carry high conical papillae. These ridges and the papillae penetrate the epithelium, but do not cause any prominences on its free surface.

The lamina propria consists of loose connective tissue with relatively thin collagenous fibers and a very few fine elastic networks; the latter do not penetrate the papillae. Besides the usual connective tissue cells, numerous lymphocytes are scat-

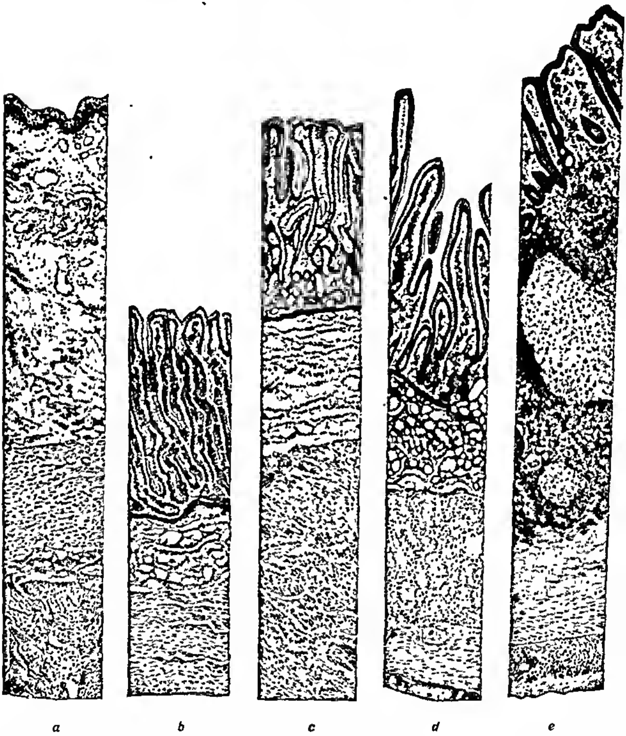

Fig. 332. Sections through five segments of the human alimentary canal. *a*, Esophagus; *b*, fundus of stomach; *c*, pylorus of stomach; *d*, duodenum; *e*, appendix. 35 ×.

tered throughout the tissue. Around the excretory ducts of the mucous glands. small lymphatic nodules are found.

At the level of the cricoid cartilage the elastic boundary layer of the pharynx is succeeded by the *muscularis mucosae.* It consists of longitudinal smooth muscle fibers and thin elastic networks. Toward the stomach the muscularis mucosae attains a thickness of 200 to 400μ and its cells have a dense arrangement.

The dense connective tissue of the *submucous layer* consists of thick collagenous and elastic networks, and small infiltrations of lymphocytes about the glands (see following text). Together with the muscularis mucosae it forms numerous longitudinal folds which cause the irregular form of the lumen in cross section. During the swallowing of food these folds are smoothed out. This is made possible by the elasticity of the connective tissue which forms the submucous layer. The outlines of the muscularis externa always remain circular.

The *muscularis externa* of the human esophagus has a thickness of 0.5 to 2.2 mm. In the cranial quarter of the esophagus both of its layers consist of striated muscle; in the second quarter the striated muscle is gradually substituted by bundles of smooth muscles; in the caudal third only the latter are found. The relations between the two types of muscular tissue are subject to individual variations The two layers of the muscularis externa are not regularly circular and longitudinal respectively; in the inner layer there are many spiral, elliptical or oblique bundles; the longitudinal muscular bundles of the outer layer in many places are irregularly arranged.

The outer surface of the esophagus is connected with the surrounding parts by a layer of loose connective tissue called the *tunica adventitia.*

Glands of the Esophagus. Two kinds of small glands occur in the esophagus: *esophageal glands proper* and *esophageal cardiac glands.* The esophageal glands proper are unevenly distributed, small, compound glands with richly branched tubulo-alveolar secretory portions containing only mucous cells. They are located in the submucous layer and can just be recognized with the naked eye as elon-

Fig. 333 Esophagus of a man; lower third; longitudinal section: *A*, Excretory duct of a mucous gland, *D*; *a*, smaller excretory duct which passes over into the secretory portion; *C*, ampulla-like dilatation, *E*, stratified squamous epithelium; *L*, collection of leukocytes in the lamina propria mucosae, *S*, *MM*, muscularis mucosae; *M*, circular muscle layer of the muscle coat. 27 ×. After Schaffer.

gated white granules (Fig. 333). The branches of the smallest excretory ducts are scarce and short, and fuse into a cystically dilated main duct (Fig. 333, *C*), which pierces the muscularis mucosae, and opens through a very small orifice. The epithelium in the smallest ducts is low columnar or cuboidal; in the enlarged main duct stratified squamous epithelium is found. The mucous glands often give

rise to cysts of the mucous membrane.

The *esophageal cardiac glands* closely resemble the cardiac glands of the stomach. Two groups of them can be distinguished: one is in the upper part of the esophagus (Fig. 334, *D*) at the level between the cricoid cartilage and the fifth tracheal cartilage; the other is in the lower part of the esophagus near the cardia (Fig. 335, *dd*). They show great

Fig. 334. Longitudinal cross section through the upper end of the esophagus with glands of the cardiac type, *D*, in the mucosa: *A*, *A'*, Openings of the glands at the apex of papillae; *E*, stratified squamous epithelium; *MM*, muscularis mucosae From an eleven-year-old girl. 27 ×. After Schaffer.

individual variations and sometimes are absent entirely.

Unlike the esophageal glands proper, they are always confined to the lamina propria mucosae. Their terminal portions are branched and curled tubules and contain columnar or cuboidal cells with a pale granular cytoplasm, which sometimes seems to give the mucin reaction; secretory canaliculi are present. The smallest excretory ducts fuse into a large excretory duct, which is sometimes cystically dilated and always opens on the summit of a papilla. Its regular, columnar epithelium often gives a distinct reaction for mucin and more or less resembles the mucous epithelium of the gastric foveolae.

In the regions of the mucous membrane which contain the upper and lower cardiac glands, the stratified squamous epithelium may be substituted in places by a simple columnar epithelium

Fig. 335. Longitudinal section through the junction of the esophagus and the stomach of man: *E*, Stratified squamous epithelium of the esophagus; *M*, stomach; *Oe*, esophagus; *S*, lamina propria mucosae; *ac*, esophageal cardiac glands; *cd*, cardiac glands; *dd*, glandular tubes; *mm*, muscularis mucosae; *u*, transition of the stratified squamous epithelium into the cylindrical epithelium of the stomach; *wd*, dilated glandular duct. 120 ×. After Schaffer.

of the same aspect as in the gastric pits. Such patches suggest erosions, that is, places denuded of epithelium. Sometimes the patches lined with mucous gastric epithelium are of considerable size and are provided with pitlike invaginations and even with tubular glands like those of the fundus; they may even contain typical zymogenic and parietal cells (see following text).

The number and development of the cardiac glands as well as of the islands of gastric mucosa in the esophagus are subject to great individual

variations. According to some investigators, the presence of this ectopic gastric epithelium may be of some importance for the origin of diverticula, cysts, ulcers, and carcinomas of the esophagus.

In many mammals, especially those which consume coarse vegetable food (rodents, ruminants, and the horse), the stratified squamous epithelium of the esophagus undergoes cornification. The esophageal glands are present in most of the mammals, but instead of being purely mucous as in man, they have a mixed character. In some species no glands are found (rodents, horse, cat).

Histogenetic Remarks. The histogenesis of the epithelium of the esophagus in man presents certain peculiarities, which are especially important in connection with the question of metaplasia, i. e., of the transformation of one epithelial type into another.

At first the entodermal layer is a simple, low columnar epithelium. It then becomes two layered and in the ninth week the superficial cells become ciliated. In the eleventh week, vesiclelike, glycogen containing elements appear between the ciliated cells, soon outnumber them, and later are transformed into squamous cells. Finally, all the ciliated cells disappear and the epithelium becomes stratified squamous. In embryos of the other mammals the epithelium does not seem to contain any ciliated cells.

THE STOMACH

In the stomach the food is thoroughly moistened, softened, and partly dissolved by the gastric juice, ground by the contractions of the muscular wall, and transformed into a pulplike mass—the *chyme.* When the chyme has attained the necessary softness it is transferred to the intestine in small portions. Thus the function of the stomach is in part mechanical and in part chemical. The first is taken care of by the external muscular coat whose different parts work in perfectly regulated coordination, the second by the various glands of the mucous membrane.

The cavity of the empty stomach in its living condition is not much larger than that of the intestine. The trumpet-shaped opening which leads from the esophagus into the stomach is called the *cardia.* To the left of the cardia the wall of the stomach forms a bulging which is directed upward—the *fundus* (or *fornix*); it continues down the right concave and the left convex margins which are called the lesser and the greater curvatures. The transition of the stomach into the duodenum, the first part of the small intestine, is called the *pylorus.* Some investigators believe the wall of the stomach is constricted in its middle part into the *isthmus.* The wall of the stomach consists of all the usual layers of the digestive tube, as outlined in the diagram on page 351.

The mucous membrane of the stomach in the living condition has a grayish-pink color, except for narrow, pale, ring-shaped areas at the pylorus and cardia. The surface of the filled stomach is stretched evenly. In the empty, contracted stomach it forms numerous high, mostly longitudinal folds. This is made possible by the loose consistency of the submucous layer and the action of the muscularis mucosae. Another much finer and more constant relief is brought about by a system of furrows, which subdivide the surface of the mucous membrane into small, slightly bulging, gastric areas 1 to 6 mm. in diameter. With a magnifying lens the surface of each area is seen to be further subdivided by tiny grooves into irregularly convoluted ridges. In a perpendicular section through the mucous membrane the furrows, which are cut across, appear as invaginations, the so-called *gastric pits or foveolae gastricae.*

The thickness of the mucous membrane in all parts of the stomach is occupied by a multitude of glands which open into the bottom of the gastric pits. The epithelium which lines the gastric pits and covers the free surface of the mucosa between them has everywhere the same structure. On the basis of differences in the glands, three portions are distinguished in the stomach.

The first zone, which forms a narrow (5 to 30 mm.) ring-shaped area around the cardia, is called the *cardiac area* and

rise to cysts of the mucous membrane.

The *esophageal cardiac glands* closely resemble the cardiac glands of the stomach. Two groups of them can be distinguished: one is in the upper part of the esophagus (Fig. 334, *D*) at the level between the cricoid cartilage and the fifth tracheal cartilage; the other is in the lower part of the esophagus near the cardia (Fig. 335, *dd*). They show great individual variations and sometimes are absent entirely.

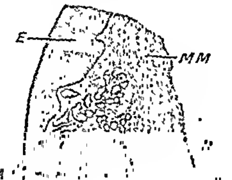

Fig. 334. Longitudinal cross section through the upper end of the esophagus with glands of the cardiac type, *D*, in the mucosa: *A*, *A'*, Openings of the glands at the apex of papillae; *E*, stratified squamous epithelium; *MM*, muscularis mucosae From an eleven-year-old girl. 27 ×. After Schaffer.

Unlike the esophageal glands proper, they are always confined to the lamina propria mucosae. Their terminal portions are branched and curled tubules and contain columnar or cuboidal cells with a pale granular cytoplasm, which sometimes seems to give the mucin reaction; secretory canaliculi are present. The smallest excretory ducts fuse into a large excretory duct, which is sometimes cystically dilated and always opens on the summit of a papilla. Its regular, columnar epithelium often gives a distinct reaction for mucin and more or less resembles the mucous epithelium of the gastric foveolae.

In the regions of the mucous membrane which contain the upper and lower cardiac glands, the stratified squamous epithelium may be substituted in places by a simple columnar epithelium

Fig. 335. Longitudinal section through the junction of the esophagus and the stomach of man: *E*, Stratified squamous epithelium of the esophagus; *M*, stomach; *Oe*, esophagus; *S*, lamina propria mucosae; *ac*, esophageal cardiac glands; *cd*, cardiac glands; *dd*, glandular tubes; *mm*, muscularis mucosae; *u*, transition of the stratified squamous epithelium into the cylindrical epithelium of the stomach; *wd*, dilated glandular duct. 120 ×. After Schaffer.

of the same aspect as in the gastric pits. Such patches suggest erosions, *that is*, places denuded of epithelium. Sometimes the patches lined with mucous gastric epithelium are of considerable size and are provided with pitlike invaginations and even with tubular glands like those of the fundus; they may even contain typical zymogenic and parietal cells (see following text).

The number and development of the cardiac glands as well as of the islands of gastric mucosa in the esophagus are subject to great individual

The Surface Epithelium. The ridges between the gastric pits and their walls are lined by a tall (20 to 40 μ), very regular, simple columnar epithelium. At the cardia it begins abruptly under the overhanging edge of the stratified squamous epithelium of the esophagus. In the pylorus it is replaced by the intestinal epithelium. In the cells on the free surface round granules fill the supranuclear part of the cells. Downward into the foveolae they become more and more confined to the free surface; in the bottom of the pits only a thin layer of granules lines the surface; these elements continue down into the neck of the glands. The granules consist of mucigen or of mucin of a peculiar type (Fig. 337). After proper fixation it can be stained with some of the dyes which are elective for typical mucus, as mucicarmine, while with other mucin dyes the result is negative. After having left the cells the granules furnish the layer of alkaline mucus which lubricates the surface of the mucosa. Unlike the mucus secreted by the mucous glands of the oral cavity, it is not precipitated by acetic acid.

In sections in which the granules of mucigen have not been preserved, the supranuclear parts of the cells appear clear and transparent and only faintly granular; the free surfaces are covered with thin terminal bars. In the midst of the clear substance a diplosome is located. A Golgi net is also present above and sometimes around the nucleus. The cytoplasm in the basal part of the cell contains threadlike mitochondria. Fat droplets and glycogen granules have been found in these cells. The introduction of glucose into the stomach does not influence the amount of glycogen in its epithelium.

Even under physiologic conditions many of the surface cells are desquamated and perish. Signs of regeneration are seen only in the deeper part of the foveolae, where mitoses are frequent in the less differentiated cells which contain but a small quantity of mucigen granules under their free surface. The newly formed cells are slowly pushed upward through growth pressure and replace the lost ones.

The Gastric Glands. These glands, which are the most important contributors to the secretion of the gastric juice, are simple, branched tubules. They are densely arranged, perpendicularly to the surface of the mucosa, and penetrate its whole thickness which measures from 0.3

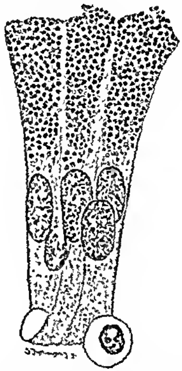

Fig. 337. Surface epithelium of the human stomach; fixed with sublimate-osmic acid and stained with gentian to show the mucous droplets. After Heidenhain.

to 1.5 mm. They open in small groups through a slight constriction into the bottom of the foveolae. The diameter of the glandular tubule is 30 to 50 μ, but the lumen is very narrow. The blind ends are slightly thickened and coiled and sometimes divide into two or three branches (Fig. 339); they almost reach the muscularis mucosae. The total number of these glands is estimated at 35,000,000.

There are four types of cells in these glands. Many different names have been proposed for them so that the nomencla-

contains glands of the same name. The second zone comprises the *fundus and proximal two thirds* of the stomach and contains the gastric glands proper or the *glands of the fundus* (named so because they were first found here). The third part, the *pyloric region*, occupies the distal one-ninth of the stomach and extends for a greater distance on the lesser curvature than on the greater; it is characterized by the presence of pyloric glands. These zones are not separated by sharply drawn limits; along the borderline the glands of one mix to a certain extent with those of the other. According to some, between the second and third zones there is a narrow strip, of some millimeters in width, occupied by a fourth type of glands, the *intermediate glands*. In the dog, the animal especially used for physiologic experimentation, this intermediate zone reaches a high development and a width of 1 to 1.8 cm.

In various other mammals the subdivisions of the stomach are much more sharply pronounced and are marked by deep constrictions which separate the organ into chambers. The esophageal, stratified squamous, sometimes cornified epithelium may invade a smaller or larger part of the stomach; this first esophageal part, as a rule, has few or no glands. In the ruminants the three first chambers, the rumen, the reticulum, and the omasus (or psalterium) are all of esophageal nature; only in the fourth portion, the abomasus, are gastric glands found, and here only does digestion occur. In the monotremes and marsupials the whole stomach is occupied by the esophageal region and the stratified squamous epithelium reaches as far as the glands of Brunner. In the pig the second or cardiac region, with mucus-secreting gastric epithelium and cardiac glands, is highly developed; the third, physiologically most important portion is the region of the corpus or fundus, while the fourth portion is the pyloric region.

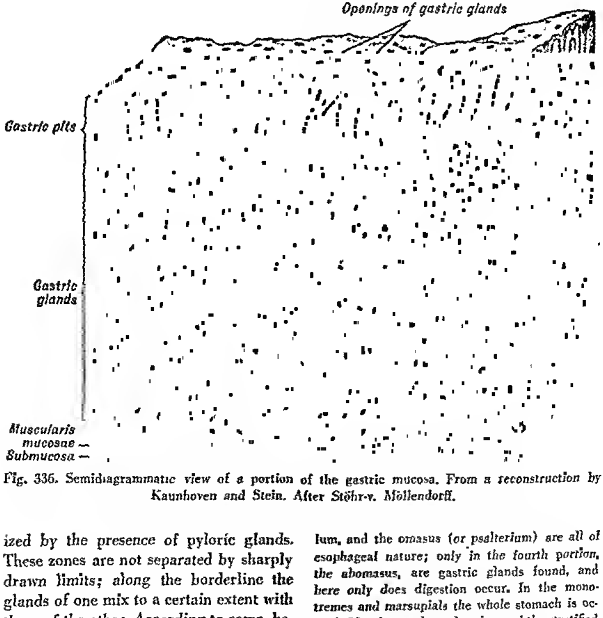

Fig. 336. Semidiagrammatic view of a portion of the gastric mucosa. From a reconstruction by Kaunhoven and Stein. After Stöhr-v. Möllendorff.

are of spherical, sometimes slightly triangular form and occupy a peripheral position between the zymogenic cells and the basement membrane. Sometimes they even cause bulgings on the outer surface of the glands, especially after prolonged activity when the zymogenic cells are small.

The parietal cell contains a large round nucleus; sometimes two or even more nuclei are present in one cell. The cytoplasm stains readily with acid aniline dyes. The cell contains a diplosome and numerous short rod-shaped mitochondria, but no distinct secretory granules. The most typical features of the parietal cells are secretory canaliculi which occupy an intracellular position and form a loose network between the surface and nucleus (Figs. 340, 341). They communicate, through a small cleft between the adajacent zymogenic cells, with a branch of the main lumen. Through this canal the secretion of the parietal cells enters the lumen.

The parietal cells do not seem to undergo any morphological changes with the various stages of functional activity. In the adult the parietal cells divide, possibly by direct division, relatively often.

Mucous Neck Cells. These are found in the neck of the glands where they are arranged in one layer and fill the spaces between the parietal cells. In passing toward the bottom of the gland they are abruptly succeeded by the *zymogenic* cells. In fresh condition they are filled with very pale, transparent granules.

In sections in which the secretory granules are not preserved and which are stained only with nuclear dyes, they are very similar to the zymogenic cells and, therefore, were overlooked by many investigators. Their nuclei, however, are different from those of the zymogenic cells because they are usually flat, sometimes concave and occupy the base of the cell. In sections stained with mucicarmine or mucihematein, the cytoplasm is filled with coarse, brightly stained granules, while the zymogenic cells remain colorless. This indicates that the mucous neck cells are mucus-secreting elements. Their mucus, however, is of a peculiar kind and its staining reactions differ from those of the mucus of the gastric surface epithelium

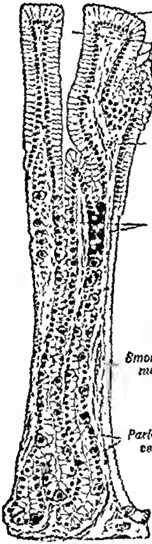

Fig. 339. Fundic glands of the stomach of a man. Zymogenic cells gray; parietal cells dark gray. 130 ×. After Braus.

and of that of the glands of the oral cavity.

Where the necks of the glands open into the narrow bottoms of the foveolae, the mucous neck cells are connected with the surface epithelium by a series of gradual transitional forms. As mitoses are not found in the mucous neck cells of the adult, it is probable that new ones arise

ture is rarely alike in any two descriptions. The four types are: (1) body chief, or zymogenic cells, (2) parietal cells, (3) mucous neck cells, and (4) argentaffine cells (of Heidenhain).

Zymogenic Cells. The zymogenic cells are arranged in a simple layer on the inner surface of the basement membrane and line the lumen in the lower half or third of the glandular tubule. After death they disintegrate almost immediately unplasm shows an alveolar structure. The spherical nucleus does not show any peculiarities. Under the nucleus in the basal part of the cell the cytoplasm contains radially striated accumulations of chromophil substance (Fig. 340); the zymogenic cells contain mitochondria and a Golgi net.

The free surfaces of the cells are provided with terminal bars. True secretory capillaries are not present. It is not clear

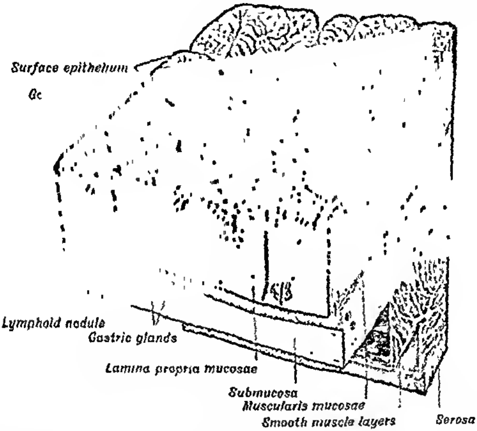

Fig 338. Surface of gastric mucosa of a man. Drawn with a binocular microscope. The cut surfaces are slightly diagrammatic. At the left the normal distribution of the gastric glands; to the right only a few are indicated Glands, gray; gastric pits, black. 17 ×. After Braus.

less there was no acid in the stomach, when they may remain for some time. In fresh condition, especially after a period of fasting, the cells are full of coarse, brilliant granules. After intense secretory activity the cells are smaller and contain but few granules near their surface. The granules are believed to contain pepsinogen, the antecedent of the enzyme pepsin. Only some osmic-sublimate and formalin mixtures preserve the granules; in most cases they dissolve and the fixed cytowhether the zymogenic cells under physiologic conditions are subject to degeneration and renewal or not. Mitoses are never found in them. It is possible that they may arise from the mucous neck cells described below, although in the adult, transitions between them—zymogenic cells with a slight mucin content near the free surface—are very rare.

Parietal Cells. Between the zymogenic cells, but more numerous toward the neck, parietal cells are scattered singly. They

mucous membrane (to one half of its thickness) and have more branches than in the body of the stomach. The glands here are also of the simple, branched tubular type, but the lumen is larger, and the tubules are coiled so that in perpendicular sections they are seldom seen as longitudinal structures. The pyloric glands contain only one type of cell; its cytoplasm is pale and contains an indistinct granulation (Fig. 343). Secretory capillaries have been described between them. The nucleus is often flattened against the base of the cell. In sections stained with hematoxylin and eosin, they sometimes resemble the mucous neck cells or even the zymogenic cells, or the cells of the glands of Brunner of the duodenum. Some investigators believe that the pyloric glandular cells are identical with the mucous neck cells, as both give similar staining reactions for mucus (Fig. 344). Certain dyes (cresyl violet, Giemsa mixture), however, seem to stain them in a specific way; they may be compared, perhaps, only with the cells of the cardiac glands. In the human stomach, the pyloric glands in the region of the sphincter may contain parietal cells. Argentaffine cells have also been described in the pyloric glands.

The Cardiac Glands. These are compound tubular glands very much like the cardiac glands of the esophagus. The terminal portions open directly into the gastric pits and show enlargements in many places. The clear glandular cells are found either alone or alternating with numerous parietal cells.

Ectopic Intestinal Epithelium and Glands in the Stomach. In the cardiac as well as in the pyloric region of the gastric mucosa, patches of intestinal epithelium and glands may be found among the gastric foveolae. Some believe them to be signs of chronic inflammation. The intestinal epithelium may be identified at once by its striated border and by the scattered goblet cells. The cardiac glands may open into these glands of Lieberkühn (see Chapter XIX).

Lamina Propria. The scanty connective tissue of the lamina propria fills the narrow spaces between the glands and the muscularis mucosae and forms larger accumulations only between the necks of the

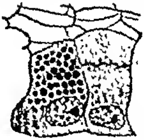

Fig. 342. Two cells from the boundary between neck and body of a gastric gland of *Macacus rhesus.* A zymogenic cell with black-stained secretory granules, and a mucous neck cell with pale vacuoles, previously occupied by mucous droplets. On the free surface a net of terminal bars. Iron-hematoxylin stain. 1000 ×. (A.A.M.)

Fig. 343. Pyloric glands from the stomach of a man. Slightly diagrammatic. 75 ×. After Braus.

glands and between the foveolae. It consists of a delicate network of collagenous and argyrophil fibrils and is almost devoid of elastic elements. Its cells have not been satisfactorily investigated. Besides oval pale nuclei which seem to belong to

through a gradual transformation of the undifferentiated epithelium in the bottom of the foveolae.

In many gastric glands the mucous neck cells advance far toward the bottom and are sometimes scattered singly between the zymogenic

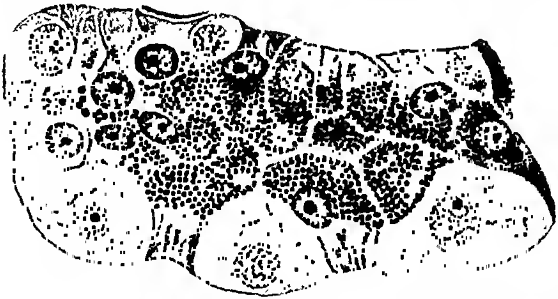

Fig. 340. Blind end of a gastric gland of a monkey (*Macacus rhesus*). Zymogenic cells with black secretory granules; between them secretory capillaries; three parietal cells with intracellular canaliculi. Iron-hematoxylin stain. 1000 ×. (A.A.M.)

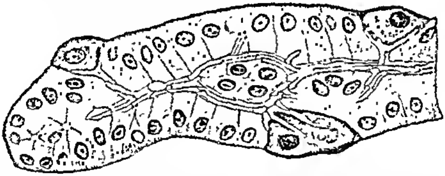

Fig. 341. Bottom of a human gastric gland with zymogenic and parietal cells. The zymogenic cells surround a narrow central canal and contain darkly stained "basal filaments" or "lamellae" (the chromophil substance). On their free surface a system of terminal bars is seen. Branches of the central lumen penetrate between the zymogenic cells as secretory capillaries; some of them reach the parietal cells and form branching canaliculi around and in their bodies. Iron-hematoxylin stain. Slightly modified after Zimmermann.

cells. This is especially prominent in the glands near the pyloric region; according to some, the glands of the narrow intermediate zone may contain only mucous neck and parietal cells and be devoid of zymogenic cells

Argentaffine Cells. Argentaffine cells, like those in the intestine (p. 401) are moderately abundant in the fundic glands and are less frequent in the pyloric glands. These cells are scattered singly, between the basement membrane and the zymogenic cells, and have a somewhat flattened form. Their cytoplasm is filled with small granules which can be stained with silver or chromium salts. They are more numerous in the duodenum. As their distribution parallels that of the intrinsic antipernicious anemia factor (p. 95) it has been suggested that they may be related to this substance.

The Pyloric Glands. In the pyloric region the foveolae reach deeper into the

stomach. The emptying of the stomach depends primarily on the contraction of the gastric musculature.

The work of all the parts of the muscular coat just described is regulated with marked precision. The wall of the stomach adapts itself to the volume of its contents without raising the pressure in its cavity.

The *serous membrane* is a thin layer of loose connective tissue, attached to the muscularis externa and covered with mesothelium. It continues into the large and small omentum.

Histophysiologic Remarks. The quantity of *gastric juice* secreted during twenty-four hours by the human stomach is estimated at 1000 to 1500 cc. The juice is a clear, colorless liquid which contains, besides water and salts, 0.4 to 0.5 per cent hydrochloric acid and enzymes. *Pepsin*, which digests protein in acid medium and clots milk, is the most important of these; it is a protein and has been crystallized by Northrop. He finds it probable "that the various pepsins vary from species to species, as do the hemoglobins." The other ferments are *rennin*, which has a stronger clotting action on milk than pepsin and is present mainly in infancy, and small amounts of a *lipase* (which splits fat). In the dog it is possible to obtain the secretion of the two main parts of the stomach separately. Whereas the body of the stomach secretes only when certain stimuli act upon the mucous membrane (for instance the ingestion of food or psychic impressions), the pyloric region secretes continuously. The secretion of the body contains both pepsin and hydrochloric acid. It is also possible to distinguish two kinds of secretion furnished by the gastric glands—the secretion of the ordinary acid gastric juice, rich in pepsin, and of a juice which is also rich in pepsin, but contains a mucin-like substance and has only a weakly acid or even an alkaline reaction It is generally believed that the pepsin is secreted by the zymogenic cells and that the granules of the zymogenic cells are prozymogen granules which are transformed into active pepsin only when they are acted upon by the hydrochloric acid. Analyses show that the pepsin content is higher, the more zymogenic cells in a given location (Linderstrom-Lang and Holter). Injection of histamine causes the secretion of large amounts of acid gastric juice low in pepsin, while stimulation of the vagus nerve results in a great increase in the pepsin content of the juice. Bowie has shown that this is accompanied by an extensive discharge of zymogen granules. It has been observed that if a small fragment of the fresh mucous membrane with gastric glands is treated with hydrochloric acid, the zymogenic cells disintegrate rapidly, while the parietal cells remain unchanged for a while. According to Ivy, the pyloric fluid does not contain enzymes in significant amounts.

The parietal cells are supposed by some to secrete a precursor of *hydrochloric acid*. Some investigators using neutral red, a dye which changes color with the increase of acidity in the medium, concluded that the parietal cells in living condition have an alkaline reaction and that they secrete an antecedent of the acid, probably a chloride of an organic base, which in the foveolae or on the surface of the mucous membrane yields the free acid. Histochemical studies show that, in the actively secreting stomach, chloride is concentrated in the connective tissue of the submucous and, to a lesser extent, of the subepithelial layers. Chlorides have not been demonstrated in any of the epithelial cells except the zymogenic ones, and in them in only small amounts.

The mucous neck cells and the surface epithelium secrete *mucus*. The gastric mucus forms a layer on the surface of the mucous membrane which is supposed by some to protect it against autodigestion by delaying the diffusion of pepsin and hy-

fibroblasts or reticular cells, the meshes of the fibers contain numerous small lymphocytes, and some plasma cells, eosinophil leukocytes, and mast cells. Sometimes, cells with *coarsely granular acidophil inclusions* are found between the epithelial cells of the glands; these are *Russell's bodies*, which may develop under physiologic conditions, but are very common in pathologic cases.

In the lamina propria, especially in the pyloric region, small, spherical accumulations of lymphatic tissue occur normally. They are sometimes called "lenticular glands." Strands of smooth muscle also occur.

The Other Layers of the Wall. The *muscularis mucosae* consists of an inner circular and an outer longitudinal layer of smooth muscle; in some places there is a third outer circular layer. From the inner layer strands of smooth muscle cells run between the glands toward the surface. The contraction of these strands compresses the mucous membrane and probably facilitates the emptying of the glands.

The *submucous layer* consists of dense connective tissue which contains fat cells and is rich in mast cells, lymphoid wandering cells and eosinophil leukocytes. This layer contains the large blood and lymph vessels and venous plexuses.

The *muscularis externa* consists of three layers—an outer mainly longitudinal, a middle circular, and an inner oblique. The outermost layer is formed by the continuation of the longitudinal fibers of the esophagus. They keep their longitudinal course only along the two curvatures, while on the anterior and posterior surfaces they gradually bend toward the larger curvature. In the pyloric region the longitudinal fibers are assembled in a layer which continues into the same layer of the intestinal wall.

The middle layer is the most regular and continuous of the three. In the pylorus it forms a thick, circular sphincter which helps control the evacuation of the

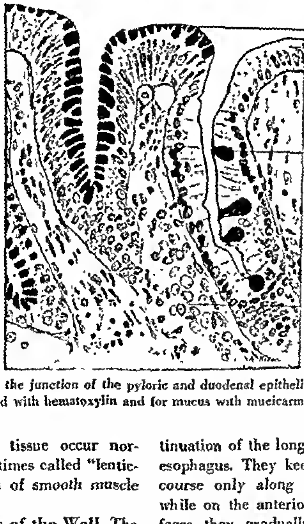

Fig. 344. Section through the junction of the pyloric and duodenal epitheliums of an eight-year-old child. Stained with hematoxylin and for mucus with mucicarmine. 220 ×.

THE INTESTINES

THE SMALL INTESTINE

The small intestine is a tube about 7 meters long and divisible into three portions, the *duodenum*, the *jejunum*, and the *ileum*, which gradually pass into one another. Their structure, although showing some differences, is everywhere the same in principle, so that one description applies to all of them. The main functions of the small intestine are (1) the forwarding of the chyme along its course, (2) the continued digestion of the chyme by means of special juices secreted by its walls and by the accessory glands, and (3) absorption of the liquefied nutritive material into the blood and lymph vessels

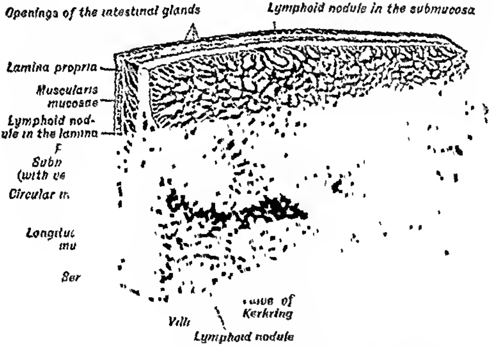

Fig. 345. Portion of wall of small intestine; drawn with binocular microscope and from sections. 17 ×. After Braus

Surface of the Mucous Membrane. In the small intestine, from which the organism receives all of its food material, the surface is enormously increased through the formation of circular folds, or *valves of Kerkring*, and the villi (Fig. 345).

The folds are constant structures and do not disappear even when the intestinal wall is distended. They begin at a distance of 2 to 5 cm. from the pylorus and reach their maximal development in the distal half of the duodenum and the proximal part of the jejunum; in the ileum they become smaller and less numerous and disappear in its middle. In the lower duodenum they reach a height of 8 mm. and usually extend over two thirds of the circumference; very often the folds branch;

drochloric acid, by inhibiting the action of pepsin, and by combining with the acid, for the mucosa is neutral during periods of inactivity. According to another opinion, autodigestion in life is prevented by an antiferment elaborated by the mucous membrane. Immediately after death autodigestion begins. It is also believed to occur if a part of the mucous membrane is damaged so that peptic ulcers of the stomach develop. But ulcers do not develop in dogs in which a portion of the mucous membrane has been removed experimentally; instead, the mucous membrane regenerates in a surprisingly short time.

The gastric mucosa also contains a substance which is necessary for the production of erythrocytes (see p. 95).

Blood Vessels and Lymphatics of the Stomach. See p. 410.

Nerves. The nerves of the stomach are of the same types and distribution as those of the intestine (see p. 412).

Histogenetic Remarks. In the very young embryo the stomach is lined by an even layer of pseudostratified columnar epithelium. In embryos of 22 8 mm., groups of tall and low cells alternate so that small pits arise, although the basement membrane remains even. In later stages (42 mm.) the pits begin to project into the underlying mesenchyme, while the tall cells between them begin to elaborate mucus. In embryos of 90 mm., at the bottom of the crypts, solid buds of granular cells appear—the primordia of the glands. In the 120 mm. stage the glandular primordia establish two kinds of cells; some of them stain intensely with eosin and are accumulated at the blind ends and later assume a peripheral position—they are the future parietal cells; others remain pale—the future zymogenic cells

At birth the length of the glands equals one half of the thickness of the mucosa. Their number gradually increases, partly through division of the blind ends of the tubes, partly through the formation of new buds of undifferentiated cells. The pyloric and cardiac glands seem to arise from the very beginning as structures different from the gastric glands.

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cous membrane, the surface of the villi and the small even areas between their bases, is simple columnar. Three types of cells can be distinguished in it: (1) columnar cells with a striated border, (2) goblet cells, (3) argentaffine cells.

The columnar cells have a prismatic form and a height of 22 to 26 μ; their outlines, however, change considerably with the movement of the villi. The free surface is covered with a striated border rich in phosphatase and the lower part of the cell contains the oval nucleus. Under the striated border there is always a thin layer of homogeneous cytoplasm. Under this layer longitudinally arranged, wavy mitochondria are accumulated (Fig. 347). The Golgi net occupies the space between these mitochondria and the nucleus. Beneath the nucleus the cytoplasm contains a group of granular mitochondria.

The bases of the cells are connected with the surface of the lamina propria. This close connection of the epithelium with the connective tissue in the living condition withstands successfully the strain arising from the movements of the villi and the mechanical action of the passing food material. However, after fixation the epithelium, as a rule, appears detached from the stroma on the summit of the villi in a continuous layer and a cavity is seen between the two tissues. This free space is an artefact, caused especially by the agonal contraction of the smooth muscles in the core of the villi.

Goblet cells are scattered between the cylindrical epithelial cells (Fig. 348). The *argentaffine cells* are more common in the glands of Lieberkühn. Everywhere in the small intestine, irregularly distributed lymphocytes can be seen penetrating from the lamina propria into the epithelium of the villi.

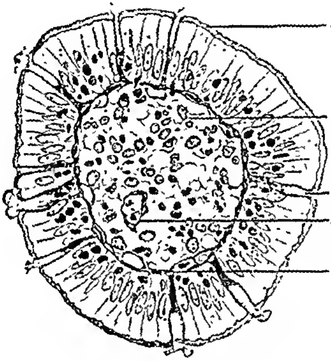


Fig. 348. Cross section of villus of human jejunum. Iron hematoxylin-azan 514 ×. Redrawn and slightly modified after V. Patzelt.

The epithelium of the villi, under physiologic conditions, is shed in considerable quantities and, together with the mucus, forms a part of the feces. In animals a short section of the small intestine may be separated from the rest of the gut and its ends connected to form a loop, while its attachment to the mesentery remains unaltered and the remaining ends of the intestine are

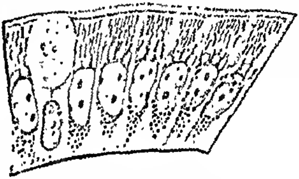
folds which run uninterruptedly around the whole intestinal tube are rare. The

Fig 316. From a longitudinal section through the duodenum of man: *A*, Artery; *BD*, Brunner's glands in the submucosa, *BD'*, Brunner's glands in the mucosa; *CM*, circular muscle cut across; *E*, mesothelium of serosa; *EM*, emptying of a Brunner gland into a crypt; *K*, crypt of Lieberkuhn; *LM*, longitudinal muscle layer; *MM*, *muscularis mucosae*; *PM*, plexus myentericus with a ganglion cell in cross section; *S*, serosa; *SM*, submucosa; *Z*, villus. 30 ×. After Schaffer.

folds are formed by all the layers of the mucosa, including the muscularis mucosae; their core is submucosa.

The *villi* are outgrowths of the mucous membrane and have a length of 0.5 to 1.5 mm. They cover the entire surface of the mucosa, on the sides and crests of the folds, as well as in the spaces between them; they give the surface a typical velvety appearance and vary from 10 to 40 to the square millimeter. In the duodenum they are wide, leaflike structures arranged with their long diameter in the *transverse direction and in alternating,* longitudinal rows; in the ileum they gradually *acquire a more finger-like form.*

Many villi, especially in the infant, are divided on their summits into two or more



g. 317. Epithelium from a villus of the small 'ine of man; fasting condition. One goblet s seen. 1000 ×. After Corti.

lobes by slits which extend for varying distances into the villi. In this way the villi are supposed to increase in number during the growth of the intestine. The innumerable openings of the glands, or *crypts of Lieberkühn,* may be seen between the bases of the villi with a magnifying lens. In perpendicular microscopical sections they have the form of simple tubes 320 to 450 μ long, which penetrate the whole thickness of the mucous membrane and almost reach the muscularis mucosae with their blind ends. Their course is perpendicular or irregularly oblique to the surface; the spaces between them are wider than those between the glands in the stomach.

The Epithelium. The *epithelium,* which covers the free surface of the mu-

cous membrane, the surface of the villi and the small even areas between their bases, is simple columnar. Three types of cells can be distinguished in it: (1) columnar cells with a striated border, (2) goblet cells, (3) argentaffine cells.

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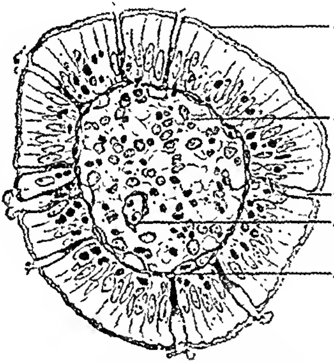

Fig. 348. Cross section of villus of human jejunum. Iron-hematoxylin-azan 514 ×. Redrawn and slightly modified after V. Patzelt.

The epithelium of the villi, under physiologic conditions, is shed in considerable quantities and, together with the mucus, forms a part of the feces. In animals a short section of the small intestine may be separated from the rest of the gut and its ends connected to form a loop, while its attachment to the mesentery remains unaltered and the remaining ends of the intestine are

sewed together. After a certain time the isolated loop is found greatly distended with digestive juices, masses of mucus and desquamated epithelium. This explains why a certain amount of feces is formed even in a starving organism. It is clear that such extensive losses require a corresponding regeneration.

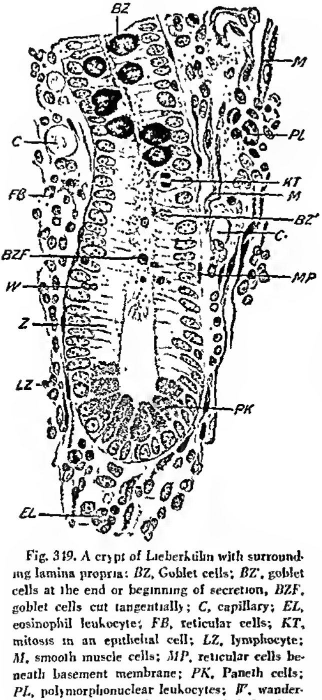

Fig. 349. A crypt of Lieberkühn with surrounding lamina propria: *BZ*, Goblet cells; *BZ'*, goblet cells at the end or beginning of secretion, *BZF*, goblet cells cut tangentially; *C*, capillary; *EL*, eosinophil leukocyte; *FB*, reticular cells; *KT*, mitosis in an epithelial cell; *LZ*, lymphocyte; *M*, smooth muscle cells; *MP*, reticular cells beneath basement membrane; *PK*, Paneth cells; *PL*, polymorphonuclear leukocytes; *W*, wandering cells in the epithelium; *Z*, epithelial cells of the gland. 380 ×. After Schaffer.

Crypts of Lieberkühn. The epithelium covering the villi continues into the glands of Lieberkühn. Above the bottom of the crypt, their walls are lined with a low columnar epithelium which contains numerous mitoses. Here regeneration takes place and the new cells moving upward differentiate into goblet cells and into the columnar cells with striated borders. All of the stages in this process are to be seen in the upper half of the crypt.

In the bottom of the glands of Lieberkühn in the small intestine, the large *cells of Paneth* occur regularly (Fig. 349. *PK*). The base of these cells is occupied by chromophil substance. Around the large, spherical nucleus are a few mitochondria. Above the nucleus the cytoplasm is filled with large, round, acidophil, secretory granules. Sometimes each granule is surrounded by a clear vacuole; however, a complete liquefaction or a discharge into the lumen is rarely seen except under the influence of pilocarpine. The granules dissolve in acids and in mineral salts, but are resistant to alkalis. The nature of these granules is not clear.

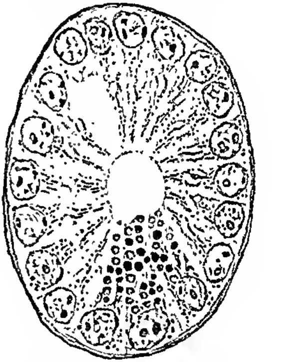

Fig. 350. Cross section of crypt of Lieberkühn of man; fasting condition. 1000 ×. After Corti

Argentaffine Cells. Between the cells lining the glands of Lieberkühn (rarely in the epithelium of the villi) are found the argentaffine cells which differ from the rest of the epithelium by their form and

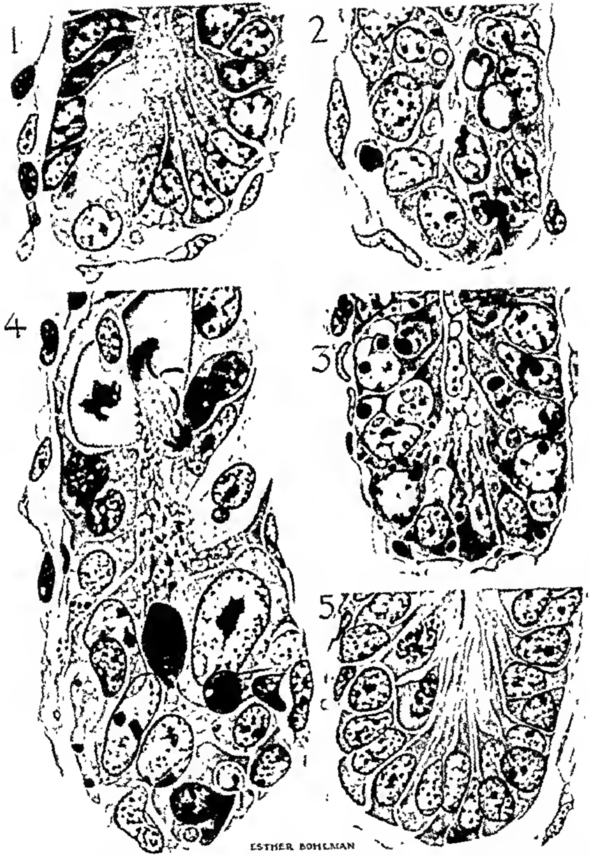

Fig. 350a Epithelium of base of crypts of Lieberkuhn from duodenum of rat, showing degenerative and regenerative changes after total body exposure to 600 r of x-rays. *1*, normal; *2*, ½ hour; *3*, 3 hours; *4*, 28 hours, and *5*, 5 days after irradiation. After M. Pierce, in Histopathology of Irradiation from External and Internal Sources. Courtesy of Atomic Energy Commission.

by the presence of specific granules in their cytoplasm (Fig. 351). They are scattered singly and their number varies greatly. Moderate numbers are found in the stomach. In the jejunum and ileum they are relatively rare; they are more common in the duodenum and particularly in the appendix. Their body, as a rule, adheres closely to the basement membrane and even bulges into its outer surface; from it a constricted continuation extends toward the lumen. The nucleus is large and usually spherical, while the oval nuclei of the surrounding epithelial cells project beyond it. The cytoplasm

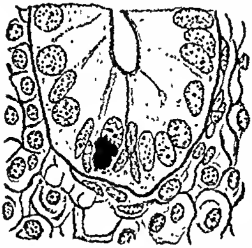

Fig. 351. Bottom of a gland of Lieberkühn, with an argentaffine cell. Redrawn from Masson.

of the base of the cells is filled with very small granules, which stain easily with eosin. They are electively impregnated and stained black by solutions of silver ammonium oxide. They acquire a brownish-yellow color after the action of chromates. They vary from species to species; they give positive tests for polysaccharides in some. The nature and function of these cells are obscure. It has been suggested that they may be associated with the intrinsic antipernicious anemia factor.

The Lamina Propria. The lamina propria of the mucous membrane fills the spaces between the glands of Lieberkühn and forms the core of the villi. It is a peculiar type of connective tissue and differs from the lymphatic tissue of the nodules of the intestine. It contains a stroma of argyrophil fibers similar to that of the lymphatic tissue. Close to the fibers are fixed cells with oval, pale nuclei; these are perhaps comparable with the primitive reticular elements of the lymphatic tissue stroma. Some of them become macrophages which may contain pigment inclusions; sometimes they react positively to tests for iron. In vitally stained animals, however, these elements contain as a rule only very few dye granules. The dye storing is more pronounced in the macrophages of the lower ileum and cecum; in these locations it is believed by some authors to be partly the result of reabsorption of the dye.

The argyrophil framework of the lamina propria at the epithelium-covered surface is condensed to a reticular basement membrane. Fine elastic networks extend from the muscularis mucosae along the blood vessels; they also surround the glands of Lieberkühn and seem to take part in the formation of their basement membrane. In addition, the argyrophil framework in many places contains strands of smooth muscle which arise from the inner surface of the muscularis mucosae, run toward the surface, and are especially prominent in the core of the villi. Here they are arranged parallel to the axis of the villus, around the central lacteal.

The meshes of the argyrophil framework contain large numbers of free cells. The most numerous are small lymphocytes; medium-sized forms also occur; large lymphocytes are rare. Very numerous are plasma cells in all stages of development. They are said to increase greatly in number during digestion. Many of them degenerate and produce Russell's bodies. Besides, the lamina propria always contains granular leukocytes; the most common among these are the eosinophil cells. The vast majority of them are regarded as eosinophil leukocytes which

migrated from the blood vessels. Sometimes, especially in the guinea pig, a few eosinophil myelocytes of local origin, with occasional mitoses, can be found. Mast cells are always present. Among them are small, young cells with but a few granules.

Many lymphocytes and a few granular leukocytes penetrate the epithelium on the villi or the glands of Lieberkühn, and occasionally even pass into the lumen. This phenomenon increases in intensity in the caudal direction and reaches its highest development in the large intestine. Mast cells are rarely found in the epithelium of the human intestine.

Another peculiar type of wandering cell found in the epithelium of the crypts in many animals is a cell with a small, round, dark nucleus and a large, swollen body containing a number of large, round granules or droplets which stain bright red with eosin—the *globular leukocyte.* These elements are small lymphocytes which elaborate acidophil inclusions in their protoplasm. Sometimes they divide mitotically; they are also found in a degenerating condition with a pyknotic nucleus.

Lymphatic Tissue. In many places the lamina propria of the small intestine contains true lymphatic tissue as the peripheral lymph nodules. Small (0.6 mm.) or large (3 mm.) isolated, spherical nodules, called "solitary follicles," are scattered all over the intestine, but are more numerous and larger in the distal part (Fig. 353); in the ileum they may be found on the surface of the *valvulae conniventes* or between them. If they are small they occupy only the deeper layer of the mucous membrane above the muscularis mucosae. Some of them may develop in the core of a villus and transform it into a club-shaped body. The larger ones occupy the whole thickness of the mucosa, bulge on its surface, and may even extend through the muscularis mucosae into the submucous layer. They are visible to the naked eye and their surface is free from villi and usually also from crypts.

Groups of many solitary follicles massed together are called *patches of Peyer* or *aggregated follicles.* They occur, as a rule, only in the ileum, but occasionally may be found even in the duodenum; their total number is estimated at 30 to 40. They always occur on the side of the intestinal wall opposite to the line of attachment of the mesentery; they are elongated, oval, slightly prominent areas. Their size differs according to the number of follicles which compose them; the long diameter varies from 12 to 20 mm., the short from 8 to 12 mm. The follicles have the same structure as those of the tonsils, and consist of dense lymphatic tissue. Their periphery is marked by a thin, capsule-like layer of condensed reticular fibers. Very often, large lymphocytopoietic or reaction centers occur in their interior.

The lamina propria and the submucosa in the vicinity of the follicles are always infiltrated with lymphocytes. Large numbers of lymphocytes penetrate the epithelium, disfigure and push aside its cells, and finally reach the cavity of the intestine. In old age the follicles and the patches of Peyer undergo involution.

The muscularis mucosae has a thickness of 38 μ and consists of an inner circular and an outer longitudinal layer of smooth muscle and of elastic networks.

The Other Coats of the Wall. The submucous layer consists of dense connective tissue with numerous elastic networks and occasional lobules of adipose tissue. In the duodenum it is occupied by a thick layer of duodenal glands; in cross section the muscularis mucosae is seen running through its glandular mass.

The external and internal layers of the muscular coat are well developed and regular in the small intestine. Carey

(1921) showed that these are not arranged, as in the conventional descriptions, in an inner circular and an outer longitudinal layer, but that the outer layer is wound as an open spiral (one turn in

Fig. 352. Section of duodenum of a child of eight years. Mucicarmine and hematoxylin stains. 65 ×.

200 to 500 mm.), and the inner as a close spiral (one turn in 0.5 to 1 mm.). Between them is the sympathetic myenteric nerve plexus (Fig. 346, *PM* and Fig. 359). Some strands of muscular cells pass from one layer into the other. The external coat consists of a layer of mesothelial cells resting on loose connective tissue (Fig. 346, *S*, *E*). At the attachment of the mesentery, the serous layer of the intestines continues onto the surface of the mesentery.

The Duodenal Glands (of Brunner). The glands of Brunner appear in the region of the sphincter of the pylorus with the first glands of Lieberkühn. Sometimes they extend into the pyloric region for several centimeters. They are arranged in lobules 0.5 to 1 mm. in diameter. Their terminal portions are richly branched and coiled tubules, which in some places may show a considerable enlargement of the lumen. These fuse into branching excretory ducts which open into the bottom or side of a crypt of Lieberkühn. The gland is located for the most part in the submucosa while the ducts pierce the muscularis mucosae. The cuboidal glandular cells contain fine granules, which stain with mucihematein after fixation in alcohol. After fixation and staining in aqueous solutions they present the usual aspect of a mucous cell—a pale, irregular cytoplasmic network with large, empty meshes and a flattened dark nucleus at the base. It is difficult to draw a distinct limit between the terminal portions and the excretory ducts, because the cells of the first gradually pass into the cells of the second. They become smaller and contain less of the mucous secretion. The transition into the crypts of Lieberkühn is very abrupt (Fig. 352).

In the distal two thirds of the duodenum the glands of Brunner gradually diminish in size and finally disappear. They show a tendency to occupy the core of the circular folds and are separated by increasing, free intervals. In some cases they seem to extend well into the upper part of the jejunum.

THE APPENDIX

The appendix is a blindly ending evagination of the cecum in man and many animals. Its wall is relatively thick, because of the extensive development in it of the lymphatic tissue which forms an almost continuous layer, with many large and small lymphatic nodules (Fig. 353, *nlnl*), as in the tonsil. The small, cleftlike lumen in cross section has an irregular, angular form. Sometimes it contains masses of dead cells and detritus, in other

cases it is obliterated. It is difficult to draw a distinct line between the normal and certain pathologic conditions in this organ. The glands of Lieberkühn radiate from the lumen; they have an irregular shape and variable length and are embedded in the lymphatic tissue. The epithelium of the surface of the glands contains only a few goblet cells and consists mostly of columnar cells with a striated border. The zone of undifferentiated cells with mitoses is shorter than in the small intestine. In the bottom of the glands, besides occasional cells of Paneth, argentaffine cells are regularly present. They are more numerous than in the small intestine and number on the average 5 to 10 to a gland; they also occur in the upper part of the glands. Villi are absent.

The lymphatic tissue of the appendix is similar to that of the tonsils. Very often it presents chronic inflammatory changes. The muscularis mucosae of the appendix is poorly developed. The submucosa forms a thick layer with blood vessels and nerves, and occasional fat lobules. The muscularis externa is reduced in thickness, but always shows the two usual layers. The serous coat is similar to that covering the rest of the intestines.

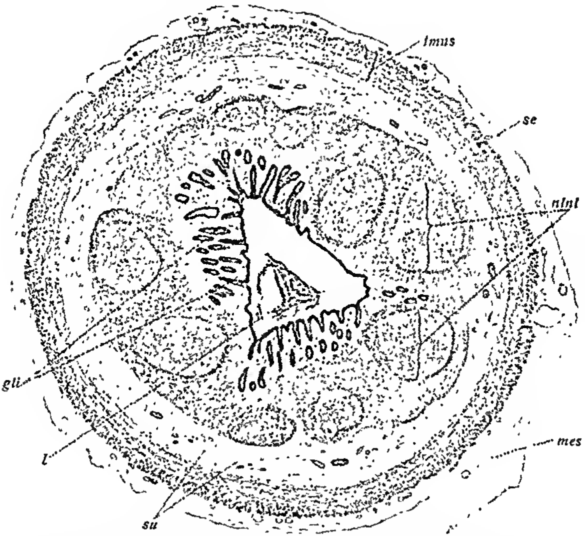

Fig. 353. Appendix from a twenty-three-year-old man: *l*, Lumen with feces; *gli*, crypts of Lieberkühn; *nlnl*, centers of lymphatic nodules; *su*, submucosa; *tmus*, muscularis externa; *se*, serosa; *mes*, mesentery. 22 ×. After Sobotta.

THE LARGE INTESTINE

The mucous membrane of the large intestine does not form folds except in its

last portion, the rectum. Being devoid of villi it has a smooth surface. The villi cease, as a rule, above the ileocecal valve.

The *glands of Lieberkühn* are straight tubules and attain a greater length than in the small intestine—up to 0.5 mm. and in the rectum to 0.7 mm.; they are arranged vertically with great regularity near one another. Their structure differs from that in the small intestine by the richness in goblet cells (Fig. 355). The free surface, between the openings of the glands, is lined with simple columnar epithelium with a thin striated border. At the bottom of the crypts are the usual, proliferating, undifferentiated epithelial cells and occasionally argentaffine cells; as a rule there are no cells of Paneth.

The structure of the *lamina propria* is essentially the same as in the small intestine; eosinophil leukocytes are abundant, often penetrating the epithelium of the crypts. Scattered solitary follicles are always present in varying numbers and are also found in the rectum. They reach far into the submucous layer. On their surface the mucous membrane usually forms an irregular invagination which is the result of fusion of the villi of the embryonic large intestine.

The *muscularis mucosae* (Fig. 355) is well developed and consists of longitudinal and circular strands. It may send slender bundles of muscle cells toward the surface of the mucosa. The submucous layer does not present any peculiarities. The muscularis externa differs from the same coat in the small intestine by the arrangement of its outer longitudinal layer, which is massed into three thick, longitudinal strands—the *taenia coli*. In the rectum it again becomes continuous

Fig. 354. Slightly tangential section through mucous membrane of human colon. The reticular fibers are condensed beneath the epithelium and about the blood vessels. The mucigen of the goblet cells stains blue. Bielschowsky-Foot and Mallory-azan stains. 600 ×.

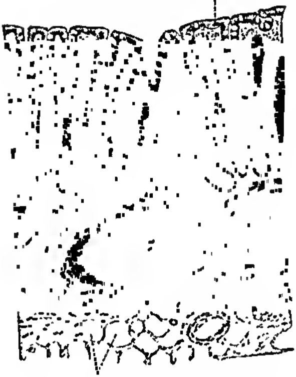

Fig. 355. Mucosa of the transverse colon of man showing a solitary follicle. 70 ×. After Braus.

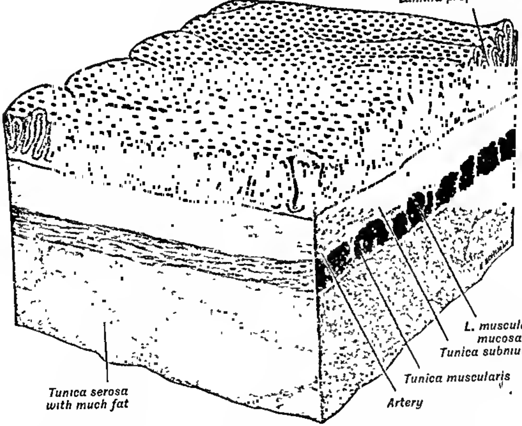

Fig. 356. Camera lucida drawing of block from human colon stained with hematoxylin. Note the single muscle layer. The openings of the glands of Lieberkuhn are clearly shown. 21 ×. Drawn by Miss E. Bohlman.

all around the periphery of the wall. The serous coat of the colon in its free portion forms the *appendices epiploicae;* these are protuberances consisting of adipose tissue and accumulations of cells similar to those in the omentum.

In the anal region the mucous membrane is thrown into longitudinal folds, the *rectal columns of Morgagni.* The crypts of Lieberkühn suddenly become short and disappear, while on the surface, along a jagged line about 2 cm. above the anal opening, stratified squamous epithelium appears with superficial, flattened cells. This is a transition zone between the mucous membrane and skin. At the level of the external sphincter the surface layer assumes the structure of the skin and here sebaceous and large, apocrine, circumanal glands appear. The lamina propria here contains convolutes of large veins, which, when abnormally dilated, appear as hemorrhoidal nodes.

Blood Vessels. The arrangement of the blood and lymph vessels in the wall of the stomach and intestine is everywhere similar in principle. The important differences depend mainly on the presence or absence of villi. Therefore, the conditions are similar in the stomach and in the colon, while the small intestine shows significant peculiarities.

In the stomach, the arteries arise from the two big arterial arches along the lesser and greater curvatures and are distributed to the ventral and dorsal surfaces. The arteries reach one side of the intestine with the mesentery. They run for a while in the serous coat and break up into large branches which penetrate the muscularis externa and enter the submucous layer, where they form a large, longitudinal, submucous plexus.

In the stomach and colon the submucous plexus gives off branches directed toward the surface; some break up into capillaries supplying the muscularis mucosae, others pierce the latter and form capillary networks in the deeper part of the mucosa; from here the capillaries pierce the mucosa almost to its surface and surround the glands with dense meshes. The capillary net is especially prominent on the surface, where it surrounds the mouths of the glands and the foveolae immediately under the epithelium.

From the superficial, periglandular capillary networks, veins of considerable caliber arise. They run downward without further anastomoses and form a venous plexus between the bottom of the glands and the muscularis mucosae. From this plexus, branches run through the muscularis mucosae into the submucosa and form here, with other similar vessels, a venous plexus, which is located nearer to the mucosa than the arterial plexus (in the dog). From the submucous plexus, the large veins follow the arteries and pass through the muscularis externa into the serous membrane. The veins of the submucous plexus (in the stomach) are provided with valves and a relatively thick, muscular coat.

In the small intestine, the submucous arterial plexus gives off two kinds of branches which run toward the mucosa and pierce the muscularis mucosae. Some of these arteries supply the crypts of Lieberkühn, ramify on the inner surface of the muscularis mucosae, and break up into capillary networks which surround the crypts in the same way as about the glands of the stomach. Other arteries are especially destined for the villi, each villus receiving one or sometimes several such small arteries. The latter enter the base of the villus and form a dense capillary network which is located immediately under the epithelium and surrounds the whole surface of the structure. Near the tip of the villus one or two small veins arise from the superficial capillary network and run downward, anastomose with the glandular venous plexus and then proceed through the mucous membrane into the submucosa where they join the veins of the submucous plexus. These veins in the intestine have no valves. Their continuations which pass through the muscularis externa with the arteries are provided, however, with valves. These structures disappear in the collecting veins of the mesentery.

Lymph Vessels. In the stomach the lymphatics begin with a well developed system of large, blindly ending or looped, lymphatic capillaries in the superficial layer of the mucous membrane between the glands. They always occupy a deeper position than the blood capillaries. They anastomose everywhere throughout the mucous membrane, surround the glandular tubules, and take a downward course to the inner surface of the mucous membrane, where they form a plexus of fine lymphatic vessels. Branches of the plexus pierce the muscularis mucosae and form a plexus of lymphatics provided with valves in the submucosa. From this submucous plexus still larger lymphatics run through the muscularis externa; here they receive numerous tributaries from the lymphatic plexus located within the muscular coat and then follow the blood vessels

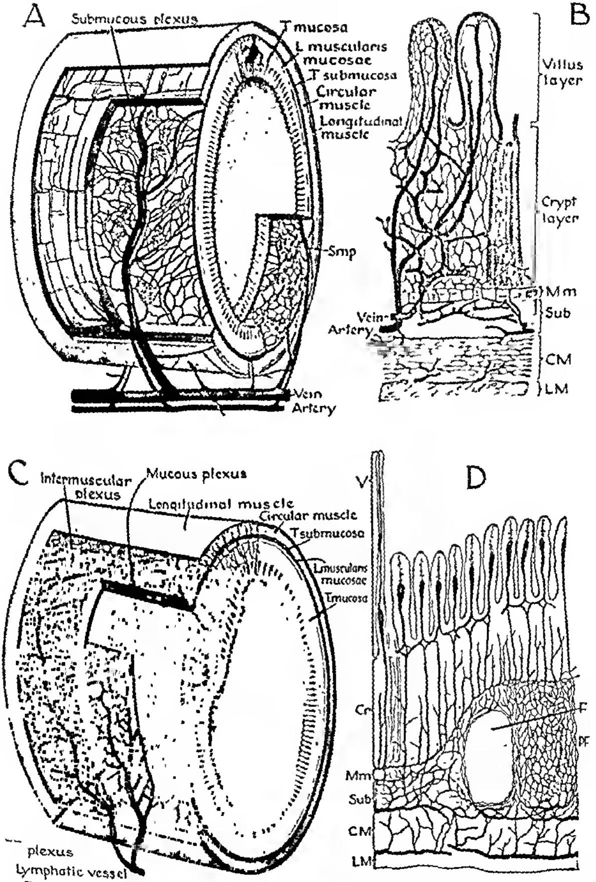

Fig. 357. Diagrams of distribution of blood vessels, *A* and *B*, and of lymphatics, *C* and *D*, in the small intestine of the dog. *B* and *D* are drawn on a larger scale to show details. *CM*, circular muscle; *Cr*, crypt; *LM*, longitudinal muscle; *Mm*, lamina muscularis mucosae; *Sub*, tunica submucosa; *V*, villus; *F*, follicle; *PF*, perifollicular plexus. Redrawn and slightly modified after Mall.

into the retroperitoneal tissues. In the wall of the colon the lymphatics show a similar arrangement.

The lymphatic vessels of the intestine play an important rôle in the absorption of fat from the small intestine. During digestion, in the living condition, all of their ramifications are seen filled with milky white lymph—a fine emulsion of neutral fats. This white lymph, drained from the intestine, is called *chyle* and the lymphatics which carry it, *lacteals*.

In the small intestine the most conspicuous parts of the lymphatic system are the *central lacteals* in the core of the villi. Each conical villus has one lacteal which occupies an axial position and ends blindly near the tip. The broader villi of the duodenum may contain two or perhaps more lacteals which intercommunicate by anastomoses. The lumen of these lacteals when distended is considerably larger than that of the blood capillaries. The wall, as seen in silver injected material, consists of very thin, large, endothelial cells with wavy outlines. This wall is everywhere connected with the argyrophil reticulum and is surrounded by thin, longitudinal strands of smooth muscle.

The central lacteals at the base of the villi anastomose with the lymphatic capillaries between the glands which have a similar arrangement as in the stomach and also form a plexus on the inner surface of the muscularis mucosae. Branchea of this plexus, provided with valves, pierce the muscularis mucosae and form on its outer surface, in the submucosa, a loose plexus of larger lymphatics. The latter also receives tributaries from the dense network of large, thin-walled lymphatic capillaries which closely surround the surface of the solitary and aggregated follicles. The large lymphatics which run from the submucous plexus through the muscularis externa into the mesentery receive additional branches from a dense, tangential plexus which is located between the circular and longitudinal layers of the muscularis externa.

Nerves. The nerve supply seems to be similar in principle in all parts of the gastro-intestinal tube. It has been especially studied in the small intestine. Here it consists of an intrinsic and an extrinsic part. The first of these is represented by nerve cells and nerve fibers located and originating in the wall of the intestine itself. The extrinsic nerves are represented by the preganglionic fibers of the vagus and the postganglionic fibers of the sympathetic. The latter run to the intestine from the celiac plexus They enter the intestinal wall through the mesentery along the branches of the large vessels.

In a section through the intestinal wall numerous nervous elements consisting of large or small groups of nerve cells and bundles of nerve fibers are seen in the narrow space between the circular and the longitudinal layers of the muscularis externa. This is the *myenteric plexus of Auerbach.* In the submucosa similar elements form the *submucous plexus of Meissner.* These plexuses form the intrinsic nervous mechanism of the intestinal wall.

In the surface view the ganglia of the myenteric plexus appear as massive, angular or star-shaped accumulations of nerve cells. They are connected with one another by thick or thin strands of nonmyelinated nerve fibers of both extrinsic and intrinsic origin. The ganglia of the submucous plexus are thin and flattened and their cells are grouped close together. They are also connected by a multitude of bundles of fibers arranged as in the ganglia of the myenteric plexus. Many varieties have been described among these nerve cells of the enteric ganglia. It is possible, however, to reduce the number of all varieties to two principal forms, which in any particular case may present differences in their secondary characters (Fig. 358). The first type occurs exclusively in the myenteric plexus. It is a multipolar cell with short dendrites which terminate in brushlike arborizations on the bodies of cells of the second type in the same ganglion. The axon enters a fiber bundle as a fine, nonmyelinated fiber; it can be traced for a considerable distance through the neighboring ganglia and fiber bundles and is supposed to form connections with cells of the second type in other ganglia. These neurons are thus of an associative nature.

The cells of the second type are far more numerous and show great variations in their forms. Their dendrites vary in number and are often missing. They divide dichotomously in the ganglia of origin or in other ganglia and terminate in diffuse receptive endings, in relation with nerve cells of the first and second types. The axon enters a fiber bundle and divides; its branches, after pursuing separate courses, terminate in the circular or longitudinal layer of the muscularis externa in the usual connection with individual smooth muscle cells. Thus the neurons of the second type are of motor nature. Those in the myenteric plexus supply the muscularis externa; those of the submucous plexus supply the muscularis mucosae and the muscles of the villi.

Besides the two kinds of cells just mentioned, a third type occurs in the enteric plexuses and also scattered in the submucosa and in the interior of the villi. This is the "interstitial cell," with a finely vacuolated protoplasm and short,

branching, varicose processes which interlace with other processes to form an irregular feltwork. It does not contain neurofibrils, so that the nervous nature of this element is doubtful. It is possibly of microglial nature.

Most of the nonmyelinated fibers of the bundles which connect the ganglia and the fibers in the ganglia are processes of the enteric neurons. The rest is formed by extrinsic fibers, mainly of vagal, and to some extent of sympathetic origin. The vagal preganglionic fibers are smooth and of uniform diameter; the sympathetic postganglionic fibers are varicose and thinner.

subserous coat and ending freely in the connective tissue.

Physiologic experiments of various kinds show that the mucous membrane of the digestive tube must be provided with sensory nerve endings. It is well known that if all the extrinsic nerves of the intestine are severed, its movements are still carried on. If the intestine is detached from the mesentery and placed in warm Tyrode solution, it will show normal peristaltic movements if the mucous membrane is stimulated by objects introduced into the lumen. This shows that the intestine is an automatic organ whose movements

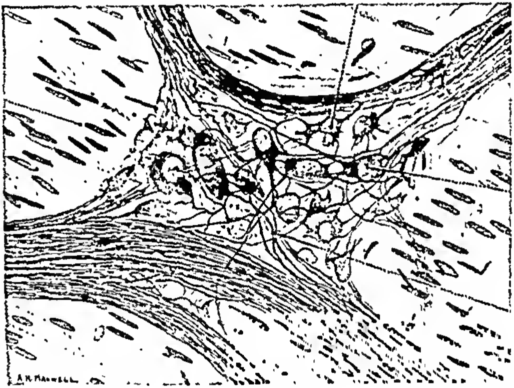

Fig. 358 Ganglion of the myenteric plexus from the small intestine of guinea pig; N_I, Neuron of Type I; N_{II}, neuron of Type II; *f tr.*, interganglionic fiber tract; *lm*, longitudinal muscle coat; *gf.tr.*, intraganglionic fibers. Silver nitrate 335 ×. After C. J. Hill.

The vagal fibers, after having entered the plexuses and taken part in the formation of the fiber bundle, terminate as pericellular arborizations on cells of the second type in the enteric ganglia. The sympathetic fibers cannot be distinguished from the axons of the motor cells in the fiber bundles. They do not seem to enter into synaptic relationship with the nerve cells of the ganglia but to take part, together with the motor axons, in the formation of the intramuscular plexuses and to terminate in connection with the muscular cells. The sympathetic fibers supply the blood vessels, too. Some of them have also been described as forming a plexus in the

are determined by the local neuromuscular mechanism and that they are only regulated through the extrinsic nerves. With the aid of modern impregnation methods numerous nerve endings of undoubtedly sensory nature have been found under and in the epithelial layers of the villi (Fig. 360, *f. ep*).

Some investigators have expressed the opinion that the enteric plexuses mediate complete reflex arcs. Their sensory component is believed to be a cell of the submucous or the myenteric plexus. Its dendrites end in the mucous membrane in contact with the epithelium of the villi or the glands of Lieberkuhn while the axons

transmit the impulse to another enteric neuron of similar nature whose axon ends in the smooth muscles. According to a widespread opinion, however, all neurons of the enteric plexuses are of efferent nature and no sensory fibers of local origin exist in the intestine. The sensory nerve endings in the mucous membrane, therefore, must be of extrinsic nature. The local reflexes in the intestine in this case are explained by the so-called "axon reflexes." The axons of the enteric neurons are supposed to divide into two branches. The stimuli are received by the end branchings of one of them and the impulse is transmitted from this branch to the other without passing through the cell body. The synaptic nature of the connection, which has been chosen as the basis for the description just given, is supported by the vast majority of authors.

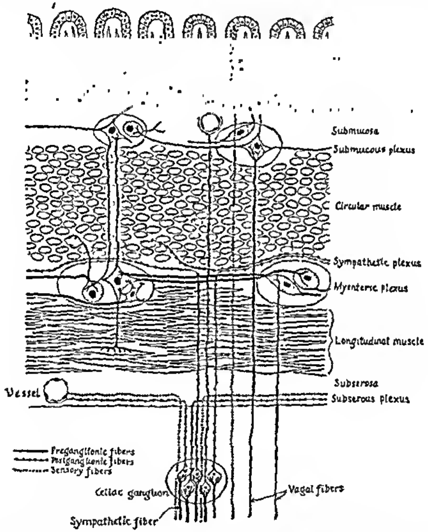

Fig. 359. Diagrammatic representation of the relations of the elements of the gut plexuses as seen in longitudinal section of the gut wall. Redrawn and modified from C. J. Hill.

Histophysiologic Remarks. An important rôle in the digestion of the chyme in the small intestine is played by the two large glands attached to the duodenum, the *liver* which secretes the bile, and the *pancreas* which secretes the pancreatic juice. The wall of the intestine itself adds an important secretion, the *intestinal juice.*

The glands of Brunner, like the pyloric glands, secrete continuously. Their secretion is a viscid, mucous, alkaline liquid. It also contains a proteolytic enzyme which is activated by hydrochloric acid and closely resembles pepsin. It is supposed to be especially active in the digestion of the collagen of adipose tissue, and thus makes the fat of the latter easily accessible to the action of fat-splitting enzymes.

The intestinal juice proper is secreted by the portions of the small intestine

which do not contain glands of Brunner. It is a yellow, alkaline liquid which always contains small flakes of mucin mixed with desquamated epithelial cells and many micro-organisms. There are several important enzymes in the juice: erepsin, which breaks down the proteins to amino acids, a lipase, a nuclease, several enzymes for splitting carbohydrates, and enterokinase which activates trypsinogen. Northrop has shown that the latter may be activated by other means. A large part of the various wandering elements which penetrate the epithelium and enter the cavity of the intestine. The mucus obviously originates from the goblet cells in the crypts and on the villi.

The *secretion of the large intestine* contains small quantities of enzymes. Its main constituent, the mucus, plays a mechanical rôle in providing the necessary consistency to the feces and lubricating the mucous membrane.

The nutritive components of the chyme are converted into soluble, easily diffusible substances so that they can be absorbed. The partially digested proteins coming from the stomach are broken down into their constituent amino acids by the action of trypsin and erepsin The neutral fat is split into glycerol and fatty acids. Some authors have described the penetration of minute fat droplets through the border of the epithelium, but this has not been confirmed. The carbohydrates are transformed into simple sugars, mainly glucose. These substances are absorbed by the surface epithelium of the in-

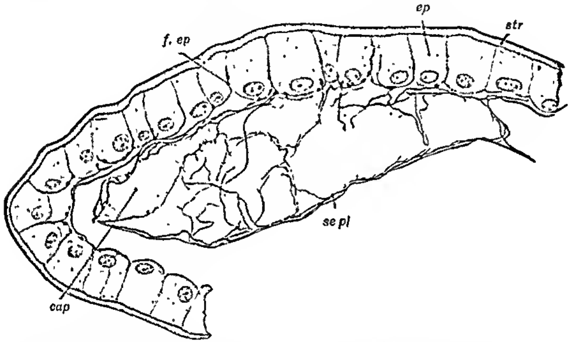

Fig. 360 Terminations of sensory fibers between the epithelial cells of a villus of a newborn rabbit. Silver method of de Castro· *ep*, Epithelium of villus; *str*, striated free border; *se pl*, subepithelial plexus; *f. ep*, fibrils which penetrate between the epithelial cells; *cap*, capillary. 560 ×. After C. J. Hill

enzymes is absorbed by the mucous flakes. The mucin of the intestinal juice plays an important rôle in the formation of feces.

Secretin is a hormone formed in the duodenal mucosa which is absorbed by the blood and stimulates the pancreas.

The surface of the villi serves primarily for absorption, while the intestinal juice is secreted by the glands of Lieberkühn. What cells of the latter are instrumental in the secretion of the enzymes is not known. It has been suggested, but not proved, that an important part in the formation of the intestinal juice is taken by

testine, especially by that of the villi. The total surface is estimated at 4 to 5 square meters.

This tissue layer acts as a barrier through which all nutritive substances have to pass before they can be utilized by the organism. The absorptive process cannot as yet be explained on the basis of known physical laws of diffusion and osmosis. After passing through the epithelium the different substances undergo complex transformations in the first layer of living cells they meet. The nature of these transformations is for the most part obscure. Many investigators have endeavored to elucidate the structural changes which take place in the isolated epithelium during fasting and absorption of different kinds of food substances, but up to the present the results are modest because of deficiencies in the histophysiologic technic now available.

The description of the intestinal epithelium, as given above, applies to the fasting, that is, to the resting epithelial cells. During absorption, especially of proteins, changes in the mitochondria have been described by some authors. The long, slender, rod-shaped mitochondria break up into granules. At the height of absorption the part of the cell body between the Golgi net below and the homogeneous layer and the striated border above may present a large number of vacuoles. Some believe the Golgi net to be the first part of the cell to show changes in connection with absorption; the lacunae enlarge and are believed to contribute to the formation of vacuoles.

Proteins can rarely be seen on their way through the epithelium since they are absorbed in the form of soluble amino acids and probably carried at once by the blood of the portal vein to the liver. However, in young, especially in suckling animals, the striated border of the epithelium seems to be much more permeable to foreign substances than in the adult. It is possible that even unchanged protein may enter by the intestinal epithelium of the young because it is adapted to the absorption of proteins with milk. Accordingly, in suckling mice, the epithelium of the small intestine, especially in its lower part, contains many large granules of more or less solid inclusions, apparently of protein nature. They may be compared with the meconium corpuscles found in human fetuses (Fig. 362). They have usually a distinctly yellow color which is believed to be due to the adsorption of bile pigment by the inclusions. Even microscopically visible particulate matter, as granules of India ink, when fed to suckling mice penetrate the epithelium, a phenomenon which has never been observed in adults.

Of all the nutritive substances, the transformation of the fat is the easiest to follow because it appears in the form of sharply outlined droplets which give characteristic staining reactions. During absorption of fat, the striated border and the subjacent homogeneous protoplasmic layer always remain free of fat droplets. The findings of observers who claim to have noted foreign fat droplets within the striated border during absorption have not been confirmed except, perhaps, for suckling animals. An increasing accumulation of fat droplets, at first small, and then larger, develops during absorption above the Golgi net and the nucleus. This shows that at least a part of the soluble constituents of the fat molecule, glycerol and fatty acids, are absorbed through the striated border and are at once synthesized by the epithelial cell into neutral fat. In what way the synthesized fat and its nonsynthesized constituents find their way into the lacteals of the villi where they appear as an emulsion, the chyle, is not known. Some investigators claim to have seen the fat droplets accumulate temporarily between the basal parts of the epithelial cells. Others have attributed an im-

portant rôle to leukocytes in the transferring of the absorbed fat from the epithelial cells into the lacteals. This is probably not correct.

The *amino acids* and *glucose* enter the blood of the subepithelial capillaries and with it they are brought through the portal vein into the liver where they undergo further transformations. The largest part of the absorbed fat is carried away from the intestine with the chyle.

If trypan blue is fed for a long time to adult mice, this dye will be absorbed only in very small quantities which are not retained by the epithelium and are excreted by the kidneys. Small blue inclusions are found only exceptionally in the epithelium. But suckling mice, whose mother has been stained with parenterally introduced trypan blue, and which, accordingly, receive slightly colored milk, soon develop a distinct blue staining of the epithelium of the small intestine. Here the above-described protein inclusions, which normally are stained yellow with bilirubin, appear blue because of adsorption of trypan blue.

If an adult rabbit or guinea pig receives trypan blue by subcutaneous or intravenous injection, the epithelium in the lower parts of the small intestine and in the colon accumulates an appreciable number of dye inclusions. In this case they are not combined with any preexisting proteins or other inclusions, but are of the same character as those found in the macrophages. This "true" storage has also been explained as being due to the reabsorption of the dye secreted from the blood into the juices of the upper part of the intestine. In suckling mice the parenteral application of trypan blue fails to produce a marked storage of the dye by the epithelium, presumably because there is not sufficient secretion of the dye in the upper part of the digestive tract. The "true" storage of the dye absorbed by the intestinal epithelium from the cavity of the intestine and especially the storage in macrophages of the stroma seems to be influenced by the presence or absence of the dye in the circulation If adult animals are only fed with trypan blue without a concomitant parenteral introduction of the dye, the dye absorbed by the epithelium is supposed to be eliminated at once into the blood which is free of dye and excreted by the kidneys. In a parenterally stained animal, the dye-containing blood is unable to take up the dye from the epithelium and the dye accumulates in the cells of the latter after sufficient time.

An important mechanism for the transmission of substances which have been absorbed by the epithelium into the blood and lymph are the movements of the villi They can be observed in a living animal (dog) if a loop of the intestine is split open and the surface of the mucous membrane is watched with a binocular microscope. There seems to be no relation between the movements of the individual villi. Every villus contracts independently, approximately six times a minute Here and there a villus is seen suddenly to become shorter by about one half its length while its thickness remains unchanged, then it

Fig. 361. Fluorescence photomicrograph of small intestine of rat during absorption of vitamin A concentrates. The vitamin A fluorescence is imparted by the epithelium, by the lamina propria of the villi, and by the contents of the lacteals After Popper and Greenberg.

expands again Thus, during the contraction, the volume of the villus is greatly reduced and the contents of its capillaries and especially of the central lacteal are forwarded into the submucous plexus. When the villus expands, the liquid which penetrates through the epithelium is believed by some to reach the central lacteal and the blood capillaries The expansion calls forth another contraction and so on; the contraction is obviously due to the shortening of the longitudinal muscular strands of the core of the villus. The movement is believed to be regulated by the submucous plexus of Meissner; direct mechanical stimulation of the base of a villus with a bristle

also calls forth a contraction; the stimulus radiates from the affected villus to the surrounding ones.

Histogenetic Remarks. The histogenesis of the mucous membrane of the intestine resembles that of the stomach. At first the boundary between the epithelium and the connective tissue is even. The development of villi begins in embryos of 20 mm. in the duodenum and gradually extends downward. In the duodenum, jejunum, and the upper part of the ileum they arise as isolated epithelial outgrowths. In the remaining parts of the intestine, longitudinal ridges develop which later are subdivided by the transverse furrows into single villi. The number of the villi in a given stretch increases through the appearance of new outgrowths in the hollows between the older villi. In a fetus of 100 mm. villi are found all along the intestine, including the colon, although they disappear from the latter in the later stages. This is due either to a fusion of the villi from their base upward or to their shortening through the stretching of the growing wall In an embryo of 55 mm. the supranuclear protoplasm of the epithelial cells on the tips of the villi acquires a transparent aspect, while on the free surface a condensed cytoplasmic layer develops Between these elements, scattered goblet cells appear.

In a fetus of four months, the epithelium of the villi has a manifold appearance In the lower parts of the small intestine the common epithelial cells with the clear supranuclear parts contain a multitude of coarse, yellow granules (Fig. 362). They are called *meconium corpuscles* and are similar to those seen in the lumen of the intestine. Their yellowish color is due to adsorption of bile pigment. Whereas some authors believe the meconium corpuscles to be material absorbed from the lumen of the intestine, others consider them as secretory material and believe that they are later eliminated into the lumen.

Between the common epithelial cells there are now many typical goblet cells (Fig. 362, *gob*). Beginning with the fourth month, argentaffine cells make their appearance (Fig. 362, *arg*). During the seventh month the cells of Paneth appear. In the human fetus they seem to occur not only in the crypts, but also on the villi.

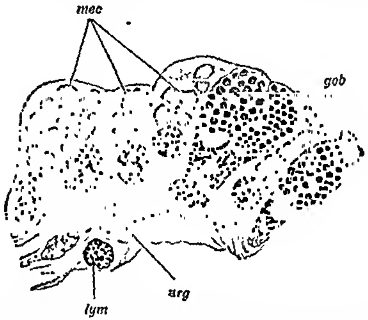

Fig. 362. Epithelium of the tip of a villus of the small intestine of a human fetus of 4½ months; 4 cells with pale, large meconium corpuscles (*mec*); 3 goblet cells (*gob*) with darkly stained droplets of mucus; one argentaffin cell (*arg*), with granules at the base, stained with eosin. In the connective tissue a lymphocyte (*lym*). 1040 ×. (A.A.M.)

The development of the glands of Lieberkühn also starts in the duodenum and proceeds downward. In a fetus of the fourth month the excavations between the villi are lined with small crowded cells with a cytoplasm which is darker than that of the epithelium of the villi. From these cells evaginations arise which penetrate the subjacent connective tissue. In the seventh month, besides the formation of glands from new invaginations, a dichotomous division of the blind ends of the existing glands contributes largely to the continuing increase of the number of glands. Bifurcation of the crypts proceeds in the newborn.

The glands of Brunner make their appearance during the sixth month as massive, epithelial ingrowths in the depth of the duodenal crypts. In a fetus of 290 mm. they are numerous in the

upper part of the duodenum and consist of branching tubules. Farther downward they are smaller and the intervals between them larger.

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Chapter XX

LIVER, BILE DUCTS, AND GALLBLADDER

THE LIVER

THE liver plays an indispensable part in the metabolism of the body and in certain digestive processes, including the elaboration of bile. The liver is the largest gland of the organism and weighs about 1.5 kg. in men and slightly less in women. It occupies the upper right quadrant of the abdominal cavity, a part of its surface being attached to the diaphragm. It arises in the embryo as an evagination of the intestine and develops into a compound gland whose secretory portions are branching and anastomosing tubules. In the lower veterbrates this condition remains throughout life but in the mammals the original architecture undergoes a complete remodeling.

Lobule of the Mammalian Liver. The mammalian liver is made up of small polygonal areas, each of which represents an architectural unit or lobule, 0.7 to 2 mm. in diameter. The periphery of each lobule is translucent and gray, while its center is brown. In man the outlines of the lobules are usually indistinct because the connective tissue partitions between them are poorly developed. In the pig, on the contrary, each lobule is completely surrounded by a layer of connective tissue and the lobulation is obvious (Fig. 363). When a freshly sectioned surface of such a liver is scraped with a knife, the soft tissue is squeezed out of the lobules and the remaining partitions give the impression of a honeycombed structure. In cirrhosis of the liver in man, the connective tissue is greatly increased and the lobulation completely distorted.

In the salivary and pancreatic glands each lobule represents a mass of glandular tissue drained by a duct of a certain order and size. The liver lobules, however, do not depend on the duct system, but on the distribution of blood vessels. This is clearly seen in microscopic sections of a liver whose blood vessels have been injected with colored masses (Fig. 365).

The lobule of the liver is a polygonal prism which in cross section has five, six, or seven sides. The diameter of the cross section is decidedly smaller than the height of the lobule. Running through the center of the lobule, in its long axis, is *the central vein* (Figs. 363, 364), while at the periphery are the branches of the [illegible] inter- [illegible] tic [illegible] a network about the portal vein and its branches.

In the past an attempt has been made to describe the liver lobule as the amount of liver tissue which surrounds and is drained by the smallest interlobular bile ducts. According to this idea the center of the liver lobule would be the structures in the periportal areas and the lobule would extend into the parenchyma of the several surrounding anatomical lobules. This theory considers only the bile excretory function of the liver and overlooks entirely the fact that the liver is predominantly an endocrine gland. It also disregards the structure of this organ as seen in those species, as the pig, in which the liver lobule is demarcated by a continuous connective tissue layer into a distinct unit. (See Pfuhl, Arey, Opie for discussions of this point.)

Blood Vessels. The principal afferent blood vessel of the liver is the portal vein. It collects the blood from the viscera of the digestive tract and from the spleen and enters the liver at the *porta* together with the hepatic artery. The liver of the mammals receives a smaller part of its blood supply from the hepatic artery. This relatively small vessel supplies the interlobular connective tissue and its contained structures and helps to nourish the parenchyma of the gland. In the living frog liver, numerous anastomoses have been seen between the terminals of the hepatic artery and those of the portal vein. The blood is drained from the liver by the two or more *hepatic veins;* these enter the inferior vena cava as it passes through the fossa for this vessel.

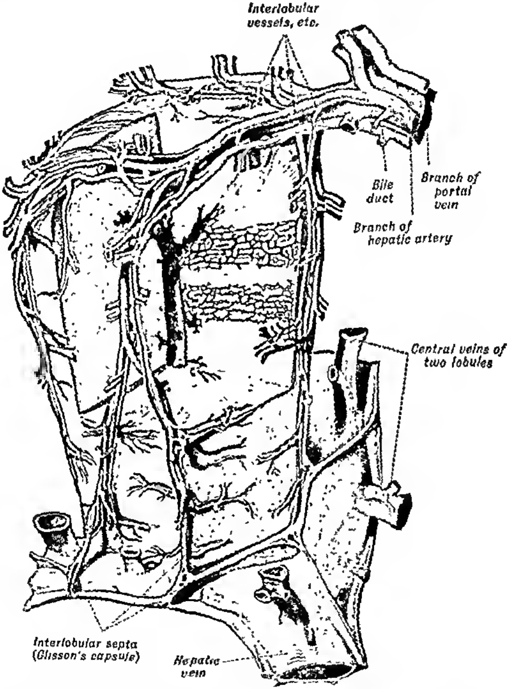

Fig 363 Lobule of the liver of a pig Wax reconstruction by Vierling. A portion of the lobule is cut away to show the bile capillaries and sinusoids 400 × After Braus

Throughout the liver the terminal

branches of the portal vein and the radicles of the hepatic vein are about equal distances apart (Fig. 366). Each radicle of the hepatic vein is surrounded by a layer of liver tissue of uniform thickness, and this mass constitutes the *hepatic lobule* (Fig. 366, *L*). Because of their central position in the long axis of the lobules, the intralobular branches of the hepatic vein are called *central veins* (Fig. 366, *C*). Several central veins join to form an *intercalated vein*—the sublobular vein of the older literature. Several of these veins unite to form a *collecting vein*; these in turn join to form the hepatic veins which pursue a course through the liver independent of the portal venous system.

Hepatic Sinusoids. The liver cell cords are separated from one another by the sinusoids of the liver (Fig. 365, *blcap*). These are irregular [illegible] spaces [illegible] lobule a[illegible] lobular portal veins with the intralobular central veins. They also receive blood from the branches of the hepatic artery. Although the direct connections of the sinusoids with both interlobular and intralobular (central) veins can be traced in sections, the connection between the hepatic artery and the sinusoids can be seen only in injection preparations and in the living animal. The finest branches of the hepatic artery empty into the sinuses at the periphery of the lobule. The contraction or dilatation of these vessels determines the amount of arterial blood reaching a sinus at any given time.

Fig 364. Low power view of a portion of liver from a twenty-two-year-old man. Two complete lobules are surrounded by portions of other lobules: *db*, Bile duct; *vint*, interlobular vein; *art*, branch of hepatic artery; *vc*, central vein; *intbd*, interlobular connective tissue; *lzb*, liver cell cords. 70 ×. After Sobotta.

The sinuses must be distinguished from capillaries (see p. 234). As seen in living animals, the lining of the hepatic sinuses appears as a continuous refractile line. which is so compact that practically no structural details can be made out within it (Fig. 368, *a*). Its cytoplasm extends as a thin film along the sinusoid. The other

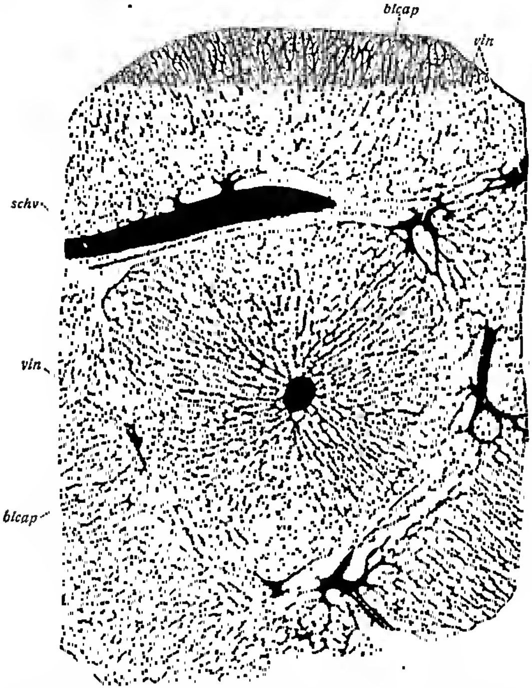

Fig. 365. Portion of the liver of a rabbit injected through the portal vein with Berlin blue and gelatin. A complete lobule surrounds the central vein; *blcap*, Hepatic sinusoids; *vin*, interlobular veins; *dbi*, interlobular bile ducts; *schv*, large interlobular vein 54 ×. After Sobotta.

As seen in sections the lining is composed of an irregular alternation of two kinds of cells connected by many intermediate forms. One of these, the *undifferentiated lining cell*, has a small dark nucleus lining cells are fixed macrophages—the phagocytic stellate cells of v. Kupffer. They are distinctly larger than the cell type just described. In microscopic sections their cytoplasm often extends into

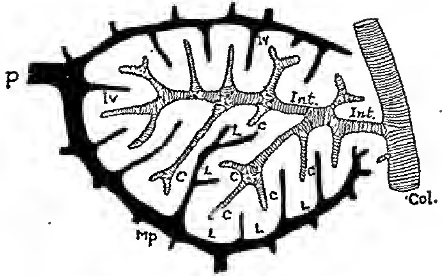

Fig. 366. Diagram showing that the branches of the portal vein (black) are separated from the radicles of the hepatic veins (cross hatched) by a uniform layer of hepatic tissue (white): *P*, Large branch of portal vein; *Mp*, medium-sized branch of portal vein; *Iv*, interlobular veins; *C*, central veins; *Int*, intercalated vein; *Col*, collecting vein; *L*, hepatic lobules. Redrawn and slightly modified after Pfuhl.

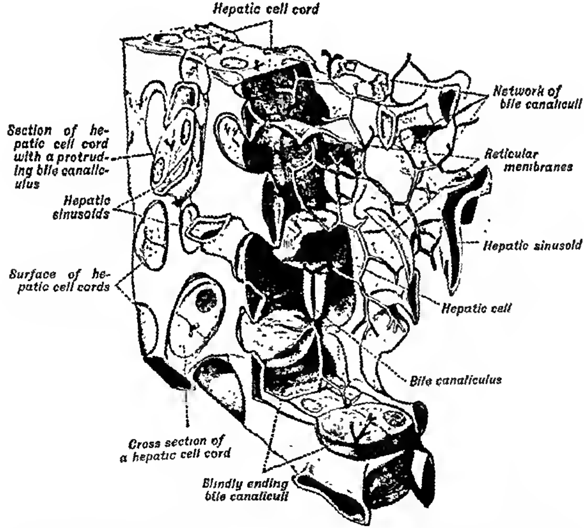

Fig. 367. Reconstruction (by A. Vierling) of a portion of a lobule of a human liver. Hepatic cell cords in yellow, with red nuclei, sinusoids, blue; bile capillaries, green. 1000 ×. After Braus.

well defined processes and one often gets the impression that these cells project into the lumen (Fig. 368, *d*). They have large oval nuclei with a small prominent nucleolus. Frequently these cells contain granules of green waste pigment, or engulfed erythrocytes in various stages of disintegration, and iron-containing granules. In animals vitally stained with the two cell types (Fig. 368, *a*, *b*, *c*, *d*). The more vital dye introduced, the more numerous and larger are the phagocytes. The increase is thought to be due to mobilization of the undifferentiated lining cells. Smooth muscle cells have been described at the junction of the sinuses with the central veins.

Marked changes occur continuously in

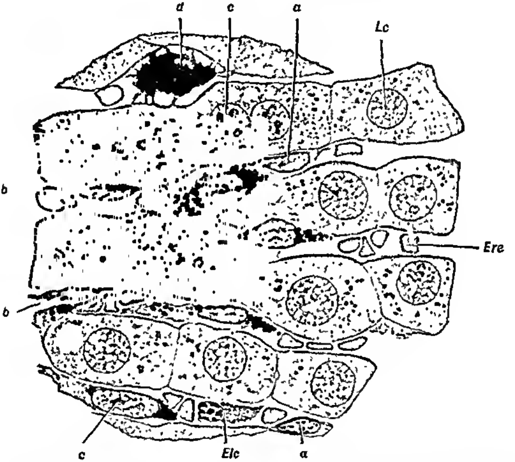

Fig. 368. Liver of rabbit, injected intravenously with India ink: *Lc*, Liver cell; *Erc*, erythrocyte in lumen of sinusoid; *Elc*, eosinophil leukocyte, *a*, cells of wall of sinusoid in resting condition; *d*, stellate cell of Kupffer; *b* and *c*, transitions from *a* to *d*. Hematoxylin-eosin-azure II stain. (A.A.M.)

lithium carmine or trypan blue they store large amounts of these dyes in granular form. The undifferentiated lining cells of the first type, however, do not store vital dyes. When finely divided particulate matter such as Higgins' India ink is injected intravenously, carbon particles are deposited in the Kupffer cells and the indifferent lining cells of the liver sinusoids. The Kupffer cells take up more of the ink. Numerous transitional forms connect

the caliber of the sinuses and in the rate of flow of blood through them. For the frog these changes have been explained as resulting from the activity of sphincters controlling the inflow and outflow of the blood of the sinuses. This mechanism permits the storage and release of blood from the liver.

Hepatic Cells. The liver cells *are arranged* more or less regularly in cords which form columns extending radially

from the central vein to the periphery of the lobule. The cords may branch slightly and anastomose with nearby cords, but in spite of this their general direction is perpendicular to that of the central vein (Fig. 364, *lzb*). Between them are broad, irregular, thin-walled sinusoids. The liver cells are polygonal in shape and have six or more surfaces. Most liver cells have one large round nucleus, although binucleated cells are not uncommon. The nucleus is quite vesicular; it has a smooth membrane and one or more very prominent nucleoli and a few small chromatin dots (Fig. 369).

Fig. 369. Section through the margin of lobule from human liver, showing the connection between a liver cell cord and an interlobular bile duct. The latter is surrounded by collagenous tissue. Bielschowsky silver impregnation and Mallory-azan stain. 720 ×. Drawn by Miss E. Bohlman.

The cytoplasm of the liver cell presents an extremely variable appearance which reflects to some extent the functional state of the cell. Both glycogen and fat are dissolved in the preparation of the usual sections but by appropriate methods glycogen and fat inclusions are readily demonstrable in them (Figs, 9, *3*, *1*; 10, 11). Their actual content of these constituents shows great variations under normal conditions. Sometimes the liver cells may be almost completely filled with glycogen, while at other times they may contain a large number of fat droplets. The content of the liver cell in protein inclusions also shows great variations. The relative amounts of these substances demonstrable in the liver cells depend primarily on the amounts of carbohydrate, fat, and protein in the diet and on the stage of digestion.

The liver cells contain a cytocentrum which may be obscured in the large cytoplasm, mitochondria of extremely variable appearance, and a Golgi net close to the nucleus or, more frequently, toward the bile capillary. There are also vacuoles in the liver cells which stain supravitally with neutral red; their relation to the Golgi apparatus is unknown. Many attempts have been made to correlate the Golgi net and the mitochondria with the various functional states of the liver. It is improbable that the mitochondria play a rôle in the storage of glycogen.

According to one author, the liver lobule in the white mouse may be divided, on differences in the mitochondria, into three zones: (1) zone of permanent repose, (2) zone of permanent function, (3) an intermediate zone. The zone of permanent repose surrounds the central vein. Here the mitochondria are fine, long, irregularly curving threads of uniform thickness with a few granular ones between them (Fig. 370, *A*). The mitochondria of this zone do not change during normal alimentation or in starvation. This zone probably constitutes a region of reserve, which becomes active only when feeding is excessive or when the adjoining parenchyma is injured The zone of permanent function lies at the periphery of the lobule (Fig. 370, *C*). Here the mitochondria always show evidences of activity. When the mice were not fed for eighteen to twenty-four hours, the mitochondria were filamentous in practically all of the cells of a given lobule except in this zone of permanent activity. The intermediate zone lies between the other two; in it the mitochondrial morphology changes

with the functional activity of the liver (Fig. 370, *B*). This zone is at rest during starvation; its mitochondria begin to function with digestion. Accordingly, mitochondrial activity proceeds from the periphery to the center of the lobule during the course of digestion.

By feeding fat the liver cells become somewhat larger and full of fat droplets. The mitochondria in this condition decrease as the fat increases. On feeding carbohydrates the cells become pale and between the glycogen granules, small, iron-containing particles and some fat droplets appear. On feeding proteins the cells become very large and full of protein inclusions.

In spite of the manifold functions which the liver cells perform, there is a marked similarity in appearance in all of them. This is at variance with what is seen in other organs, in which highly specialized functions are carried on by cells which morphologically are highly differentiated. It would appear that all of the liver cells are equally endowed with the same functional capacities, but that their active participation in these processes under normal conditions depends on the location of the cell in the lobule.

Bile Canalicules. In adult man the liver cell cords in cross section consist of but two adjacent cells between which runs a thin bile canalicule. It is a condensation of the membrane of the hepatic cells and requires special methods for its demonstration. The bile canalicules run through the length of the liver cell cord and receive short lateral branches which extend between the sides of adjoining liver cells. In planes running parallel to several adjacent cords, the bile canalicules of adjoining cords frequently anastomose with one another. The canalicules are always intercellular; this has been verified by studies on the liver of living frogs. In some species, the canalicules are stained by the methods for demonstrating phosphatase.

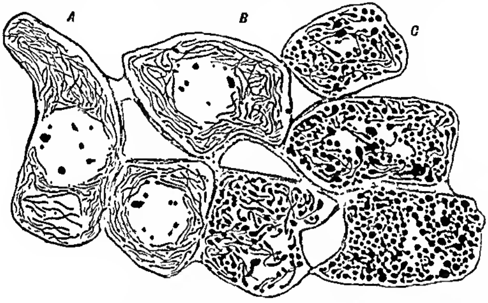

Fig. 370. Cells from the liver of a white mouse fed proteins two hours previously, showing differences in mitochondria: *A*, Cell from the zone of permanent repose; *B*, cells of the variable zone; *C*, cells from the zone of permanent function. 1200 ×. After Noel.

Connective Tissue of the Liver. The lobules of the liver are partially separated by the very thin strands of dense connective tissue called periportal connective tissue. This is a part of *Glisson's capsule*, the dense connective tissue sheathing the intrahepatic portions of the portal vein, bile duct, and hepatic artery, and is also continuous with the thin layer of connective tissue of the peritoneum covering the liver. In the human liver it is normally small in amount and barely suffices to form a framework for the interlobular artery, portal vein, bile ducts, and lym-

phatics. (See Fig. 232.) In chronic inflammatory conditions the connective tissue may be increased in amount and may show an accumulation of lymphoid cells and macrophages. In sections stained for collagenous and reticular fibers by Mallory's aniline blue mixture or with one of the silver impregnation methods, the intralobular reticular fibers become visible. The periportal collagenous connective tissue continues directly into the dense network of reticular fibers which surrounds the sinusoids. Of the latter fibers, the larger ones, as a general rule, run parallel

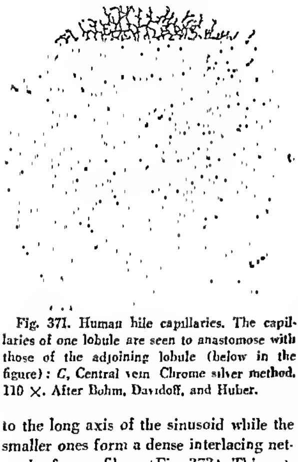

Fig. 371. Human bile capillaries. The capillaries of one lobule are seen to anastomose with those of the adjoining lobule (below in the figure): *C*, Central vein Chrome silver method. 110 ×. After Bohm, Davidoff, and Huber.

to the long axis of the sinusoid while the smaller ones form a dense interlacing network of cross fibers (Fig. 373). This network of fibers supports the liver parenchyma.

It is quite likely that the reticular fibers with their ground membrane (Fig. 47), together with the lining and Kupffer cells, form a complete wall for the sinusoids (Fig. 373). This lining completely separates the blood from direct contact with the liver cells and the space between this membrane and the hepatic cells is available in the interior of the lobule for the transfer of lymph.

Lymph Spaces. In the liver the site of origin of the lymph and its mode of entry into the periportal lymphatics are unknown. As seen in ordinary sections, the liver cells are in intimate contact with the thin lining of the sinusoids on one side and the bile capillaries on the opposite side. According to some estimates, one third to one half of all the lymph of the body originates in the liver, and yet the lymphatic vessels begin in the periportal connective tissue about the terminal ramifications of the portal vein (Fig. 374). Lymphatic capillaries have not been demonstrated within the liver lobule. Accordingly, it has been assumed by some that a *potential lymphatic space* exists between the sinusoidal lining and the liver cells (see above paragraph). This space cannot be demonstrated by injection methods.

Regeneration. If portions of the liver are removed by surgical intervention so that only about 30 per cent of its substance remains, in four or five weeks, in the rat, it will have returned to approximately its normal size. In dogs, after six to eight weeks, a section of the regenerating liver looks like one from the normal gland. Each lobule buds into a new lobule; the increase in the number of hepatic cells necessary for the bud takes place by mitosis. To a small extent it is probable that some of the newly formed liver cells arise, as in the embryo, by differentiating from the interlobular bile ducts.

Histophysiologic Remarks. No structural characteristics of the liver cells have been correlated constantly with the excretion of any of the biliary constituents. All of the hepatic epithelial cells seem to have the same abilities.

The liver plays an important part in the intermediate metabolism and storage of carbohydrates, in the metabolism of fats and of amino acids, and in the synthesis of proteins. It serves as a depository for numerous vitamins, enzymes, and hormones. The number of chemical syntheses carried out by the liver is large. It secretes bile into the the bile passages, synthesizing the bile salts and excreting the bile pigments elaborated elsewhere from the hemoglobin of destroyed red blood cells. It is rich in fixed macrophages—the Kupf-

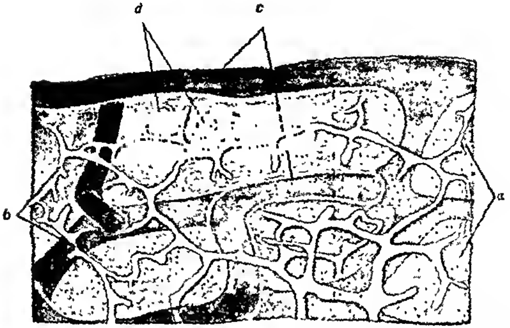

Fig. 372. Portion of liver of a living frog. Natural injection with fluorescein, as seen by ultra violet light: *a*, Liver cell cords; *b*, bile capillaries; *c*, sinusoids; *d*, indications of nuclei of hepatic cells. 600 ×. Redrawn and slightly modified after Ellinger and Hirt.

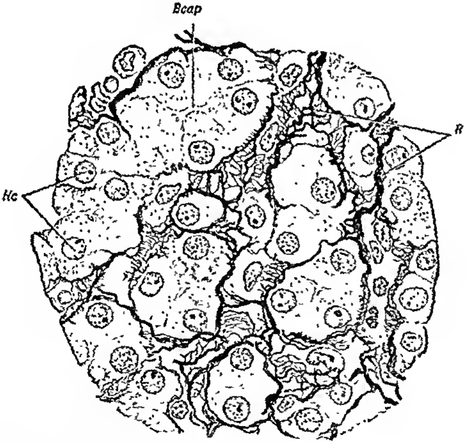

Fig. 373. Section of the liver of a man *Hc* Hep...
forming a continuous membrane on the surf
Foot stain. 840 ×. (A.A.M.)

fer cells—which have functions similar to those of macrophages elsewhere in the body. Changes in the caliber of its vessels make the liver an important storehouse and regulator of the circulating blood.

When the liver is removed by ordinary surgical methods the animals die in a few minutes. However, when a collateral circulation has developed as a result of preliminary operations, dogs may survive *hepatectomy for thirty-six to forty hours* if they are injected frequently with sugar solutions.

In contrast to the other glands of internal secretion which elaborate potent hormones, the endocrine functions of the liver are concerned with the storage of various foodstuffs and of the antianemic factor necessary for red blood cell formation. Heparin is stored and perhaps made *in the liver. Fibrinogen is made in the* liver and given off to the passing blood.

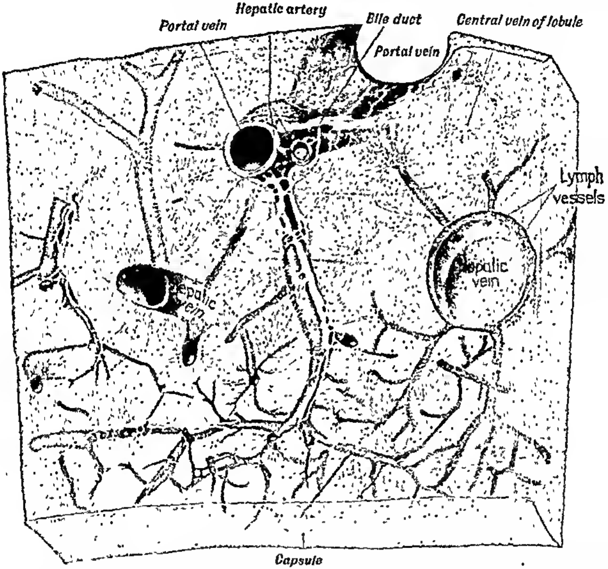

Fig. 374. Thick section of a liver of an adult cat; cleared in oil of wintergreen. The lymphatic network appears pale and the blood vessels dark. The lymphatic vessels are confined to the interlobular connective tissue, where they surround the branches of the larger blood vessels and bile ducts. 16 ×. After F. C. Lee.

[illegible]

and decreases during fasting; in this condition it may even disappear completely. Glycogen is present in submicroscopic particles; the granular appearance in sections is due to the fixation. When gly-

cogen is stored in increasing amounts in the liver cells, it is seen at first in the cells around the central vein; when sufficient carbohydrate food is eaten all of the liver cells may take up glycogen. The liver gives up its glycogen in the reverse order, that is, the cells at the periphery of the lobule are the first to give up this substance. It is claimed that in mice the first site of deposition and removal of glycogen is about the central vein.

Another important function of the liver is the *formation of urea* from ammonium carbonate. The stages of this process are not demonstrable under the microscope.

The liver cells contain much fat but an estimate of the amount present cannot be determined by microchemical-histologic methods, for a good deal of it may exist in a masked form in the liver. Under some pathologic conditions the liver cells may take up so much fat that most of the other constituents of their protoplasm are obscured, and yet an individual with such a liver may present no evidence of hepatic insufficiency.

Bile, the external secretion, is apparently elaborated continuously. It contains water, bile pigments, bile acids, cholesterol, lecithin, neutral fats and soaps, inorganic salts, and traces of urea. The bile receives, from the epithelium of the bile ducts and possibly from the neck of the gallbladder, a mucinous nucleo-albumin. Bile pigment (from broken-down erythrocytes) is formed outside the liver cells. The *bile acids* are formed in the liver cells for, if the liver is extirpated, no trace of bile acids can be found in the blood or urine. It is probable that cholesterol is not formed in the liver. When the excretion of bile is interrupted by mechanical obstruction of the bile ducts, bile continues to be formed and is absorbed from the liver at first through the lymphatics and later also by the blood vessels of the liver. When the bile pigment reaches a concentration in the blood and tissues sufficient to stain the entire body yellow the condition is known as *jaundice*. It may also be produced through the action of certain blood-destroying agents. Pure bilirubin is not toxic; the bile acids are. Occlusion of the common bile duct causes a great disturbance in the digestion and absorption of fats owing to the absence of bile acids from the intestine. After certain dyes are introduced into the organism, they may be found in the bile. If a bit of liver be teased at an appropriate time after the injection of sodium sulfindigotate, the bile capillaries will be beautifully demonstrated.

BILE DUCTS

The biliary passages are composed of an intrahepatic and an extrahepatic portion. The former consists of the intercellular (intralobular) bile canalicules and the interlobular bile ducts of the first and second orders. The interlobular ducts of the right and quadrate lobes form the right hepatic duct, while those of the left and caudate lobes form the left hepatic duct. The right and left hepatic ducts fuse and form the common hepatic duct; this receives the cystic duct, after which it continues to the duodenum as the common bile duct.

The constituents of the bile are emptied into the bile canalicules which communicate with the interlobular bile ducts by the canals of Hering (Fig. 369). The finest radicles of the bile ducts are 15 to 20 μ in diameter and have a relatively small lumen surrounded by cuboidal epithelial cells. The cells lining the ducts do not have a cuticular border. Their cytoplasm rarely contains fat droplets. The cells show an occasional mitosis. These small ducts lie on a basement membrane which is immediately surrounded by dense collagenous bundles.

The interlobular bile ducts form a richly anastomosing network which

closely surrounds the branches of the portal vein. In progressing toward the porta, the lumen of the ducts becomes gradually larger, while the epithelium becomes taller (the ducts of the second order) and has a layer of mitochondria at the base of the cell and another near the free border. These cells contain large numbers of fat droplets and, when these are very numerous, cholesterol crystals. Although a faint thickening of the periphery of these cells may be seen in some animals, it is not found in man. Lymphocytes are frequently seen migrating through the epithelium into the lumen. As the ducts become larger the surrounding layers of collagenous connective tissue become thicker and contain many elastic fibers. At the transverse fossa of the liver, the main ducts from the different lobes of the liver fuse to form the hepatic duct.

Fig. 375. Tip of a fold of the mucosa of a human gallbladder, with migrating lymphocytes in the epithelium. The blood vessels in the lamina propria are collapsed. 500 ×. Courtesy of B. Halpert.

The epithelium of the extrahepatic ducts is tall columnar. The mucosa is thrown into many folds and is said to yield an atypical variety of mucus. The scanty, subepithelial connective tissue contains large numbers of elastic fibers and some wandering lymphoid cells and occasional leukocytes; many of these penetrate the epithelium and pass into the lumen. Scattered bundles of smooth muscles first appear in the common bile duct; they run in the longitudinal and oblique directions, and form an incomplete layer around the wall of the duct. As it nears the duodenum, the smooth muscle layer of the ductus choledochus becomes more prominent and its intramural portions function as a sort of sphincter in regulating the flow of bile (see following text).

THE GALLBLADDER

The gallbladder is a pear-shaped, hollow viscus closely attached to the posterior surface of the liver. It consists of a blindly ending fundus, a body, and a neck which continues into the cystic duct. Normally it measures approximately 10 × 4 cm. in adult man and has a capacity in most animals of 1 to 2 cc. per kilogram of body weight. It shows marked variations in shape and size, and is frequently the seat of pathologic processes which change its size and the thickness of its wall. The mucosa is easily destroyed, so that in most specimens removed even a short time after death, large areas of epithelium are found to be desquamated or disintegrating.

The wall consists of the following layers: (1) a mucous layer consisting of a surface epithelium and a lamina propria, (2) a layer of smooth muscles, (3) a perimuscular connective tissue layer, (4) a serous layer, covering a part of the organ. The *mucous layer* is thrown into frequent *folds.* The major folds are subdivided by many smaller folds; they are easily seen in the contracted or even partially distended organ. But when the viscus is greatly distended, its wall becomes much thinner and most of the folds disappear although some of them can always be seen (Fig. 377).

The epithelium consists of tall columnar cells with oval nuclei, containing a few scattered chromatin granules, toward the base of the cell (Fig. 375). The cytoplasm stains faintly with eosin. A striated border, which is so characteristic in the intestinal epithelium, is lacking here although it has been frequently said to occur. Occasionally, with appropriate technic, neutral fat and other lipins may be demonstrated in the cell bodies. Mitochondria have been demonstrated in two zones of these cells as in the epithelium of the bile ducts. Goblet cells do not occur. Except in the neck of the viscus, there are no glands in its mucosa.

In the lamina propria and in the perimuscular layer near the neck of the gallbladder are relatively simple tubuloalveolar glands. Their epithelium is cuboidal and clear, and the dark nuclei are compressed at the base of the cell. They thus stand out sharply against the darker, tall columnar epithelium of the gall-

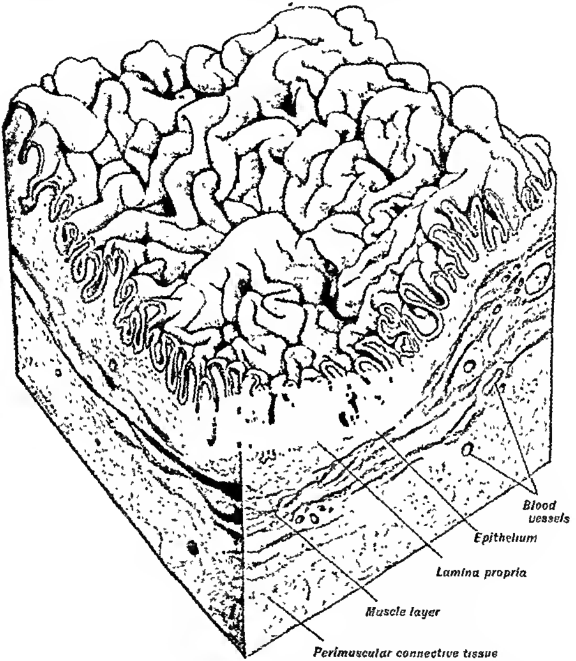

Fig. 376 Camera lucida drawing of a block from human gallbladder. Stained with hematoxylin. 32 ×. Drawn by Miss E. Bohlman.

bladder. These glands are said to secrete mucus.

Peculiar small diverticula or outpouchings of the mucosa have sometimes been confused with glands. These outpouchings are lined with and are continuous with the surface epithelium and extend through the lamina propria and the muscular layer; they are frequently slightly dilated at their blind ends. These are the *Rokitansky-Aschoff sinuses* and probably are indicators of a pathologic change in the wall of the organ which thus permits an evagination of the mucosa through the enlarged meshes of the muscular network. They are not found in embryonic gallbladders and should not be confused with the "true" ducts of Luschka described below, for the latter never communicate with the lumen of the gallbladder.

The next layer of the wall is composed of an irregular network of longitudinal, transverse, and oblique smooth *muscle fibers*. These are accompanied by a network of elastic fibers. The spaces between the bundles of muscles are occupied by collagenous, reticular, and some elastic fibers, with a sprinkling of fibroblasts. The blood vessels and lymphatics which are contained in the perimuscular layer send branches into and through the muscular layer to the mucosa.

Under the muscular layer is a fairly dense *connective tissue layer* which completely surrounds the gallbladder and is in places continuous with the interlobular connective tissue of the liver (Fig. 377). It contains many collagenous and a few elastic fibers and scattered fibroblasts with a few macrophages and lymphoid wandering cells, small lobules of fat cells, and the blood vessels, nerves, and lymphatics supplying the organ.

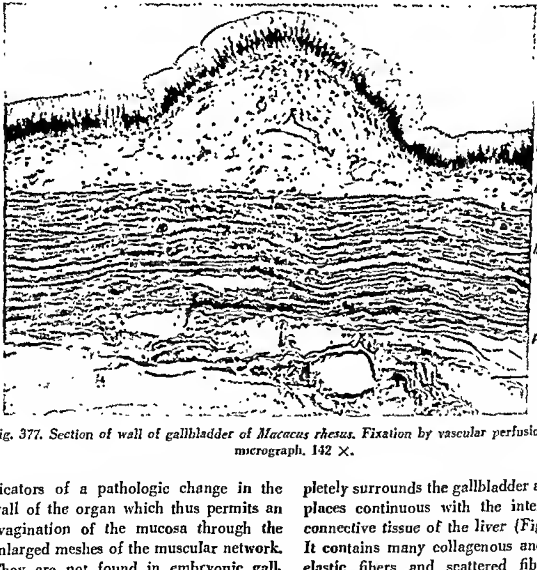

Fig. 377. Section of wall of gallbladder of Macacus rhesus. Fixation by vascular perfusion. Photomicrograph. 142 ×.

Not infrequently, particularly in the hepatic surface and near the neck, are peculiar, ductlike structures. *They may be traced for considerable*

distances in this connective tissue layer and some of them have been shown to be connected with the bile ducts. They are never connected with the lumen of the gallbladder and are probably aberrant bile ducts which have been laid down during the embryonic development of the biliary system. They have been called "true" *Luschka ducts* to distinguish them from epithelial outpouchings of the *mucosa* (see above).

The portion of the gallbladder not attached to the liver is covered with the peritoneum. Through it the ramifying arteries, veins, and lymphatics can be seen with the unaided eye. This *serosal layer* is continuous with that covering the liver.

The gallbladder at its neck continues into the cystic duct. The wall of the latter is thrown into prominent folds which constitute the *spiral valve of Heister*. These are said by some to contain smooth muscle bundles. These folds are thought to prevent distention or collapse of the cystic duct when the latter is subjected to sudden pressure.

Blood Vessels. The gallbladder is supplied with blood by the cystic artery. The venous blood is collected by veins which empty primarily into capillaries of the liver and only secondarily into the cystic branch of the portal vein. A prominent feature of the gallbladder is its rich supply of lymphatic vessels of which there are two main plexuses, one in the lamina propria (but not within the rugae) and the other in the connective tissue layer. The latter plexus receives tributaries from the liver, thus affording an explanation for hepatogenous cholecystitis. These plexuses are collected into larger lymphatics which pass through the lymph node or nodes at the neck and then accompany the cystic and comon bile ducts They pass through several lymph nodes near the duodenum and finally communicate with the cisterna chyli

Nerves. The nerves are branches of the splanchnic sympathetic and the vagus nerves. The effects of stimulation of these nerves have given rise to contradictory results in the hands of different investigators. It is probable that both excitatory and inhibitory fibers are contained in each of them. Of greater clinical importance are the sensory nerve endings, since overdistention or spasms of the extrahepatic biliary tract inhibit respiration and set up reflex disturbances in the gut tract.

Histophysiologic Remarks. The gallbladder serves as a reservoir for bile which is probably excreted by the liver continuously, if at different rates. Ingestion of fat or meat automatically discharges this reservoir. After a standard meal of egg-yolks, for instance—a maximal stimulus—three-fourths of its contents are expelled within the first forty minutes (mean figure for 76 individuals). This rate is not diminished in old age, but before puberty the male gallbladder empties more rapidly, and after puberty more slowly than the female, which maintains a constant rate.

The prevalent view that bile is expelled by the gallbladder musculature is supported by such facts as the occurrence of rhythmic contractions in isolated strips of the gallbladder wall, by the rise of intravesical pressure after ingestion of food, by the change in shape which the gallbladder undergoes when it begins to empty and by the fact that it responds to intravenous injection of *cholecystokinin*—a secretin-like substance that has been extracted from the mucosa of the small intestine.

Of special clinical importance is the inspissating function of the gallbladder. Not only does its mucous membrane withdraw water and inorganic ions from the bile but it will concentrate substances in it that are opaque to the *x*-ray—such as the halogen salts of phenolphthalein (Graham-Cole test). Failure to visualize the gallbladder after this test indicates that the organ is diseased or occluded. Whether, under normal conditions, it will absorb more than negligible amounts of other constituents of the bile has never been demonstrated. But if the mucosa be damaged it may lose its concentrating power or become semipermeable. Undoubtedly, absorption of bile salts under such conditions is an important factor in the precipitation of gallstones; and after obstruction of the cystic duct the bile may be

resorbed *in toto* or replaced by "white bile," a colorless fluid consisting largely of exudate and mucus.

Its varying "physiological capacity" in different species depends upon its anatomical capacity, its concentrating power, the total output and concentration of hepatic bile and the sphincteric resistance exerted at the choledocho-duodenal junction.

There is a little evidence in favor of a secretory function of the gallbladder. A variety of mucus is added to the bile as it passes down the larger bile ducts and mucus-secreting glands are fairly numerous in the neck. But the greater part of the gallbladder epithelium does not secrete mucus.

In a few animals, a gallbladder is never present. Its surgical removal in man is often followed by a marked dilatation of the biliary passages.

THE CHOLEDOCHO-DUODENAL JUNCTION*

In man this zone comprises the portion of the duodenal wall that is traversed by the ductus choledochus and pancreaticus and by the short ampulla into which they usually empty. For most of its length it consists of an oblique passage through the submucosa of the plica longitudinalis, but proximally it is guarded by a contractile "window" in the muscle of the duodenum, and distally by the valvules of the ampulla of Vater. From "window" to ostium, associated bile and pancreatic passages are invested by a common musculus proprius, called the sphincter of Oddi.

Fig. 378. Transverse section of the plica longitudinalis of a 43 cm. human fetus. 31 ×. After Boyden. Surgery, 1937.

* This section was contributed by E. A. Boyden.

The Fenestra Choledocha. Upon removing the ducts the aperture in the intestinal musculature is seen to resemble a "gridiron incision"—a lengthwise hiatus in the longitudinal layer being superimposed upon an obliquely transverse window in the circular layer, the two being somewhat camouflaged by *"reinforcing fibers"* and by *"connecting fibers"* that pass to the ducts from the margins of the aperture. The shape and size of the latter determine the gauge of calculi that can enter the wall and the degree to which duodenal tone and peristalsis will interfere with the passage of bile. As soon as the ducts enter this "window" they begin to taper.

The Sphincter of Oddi. This muscle varies greatly in different species. In man it may consist of four parts: (1) *The sphincter choledochus* (Fig. 378), a strong annular sheath a centimeter or less in length, which invests the common bile duct from just outside the fenestra to its junction with the pancreatic duct; (2) *the fasciculi longitudinales*, anterior and posterior longitudinal bundles which cover the interval between the two ducts and extend from the margins of the fenestra (or from the extra-duodenal portion of the ducts) to the ampulla; (3) *the sphincter ampullae*, a delicate meshwork of fibers about the ampulla of Vater (if present) and strongly developed in only one-sixth of adults; and (4) *the sphincter pancreaticus*, present in one-third of adults as a band encircling the pancreatic duct just before it joins the ampulla. The first is so placed as to stop the flow of bile (thus causing the gallbladder to fill dur-

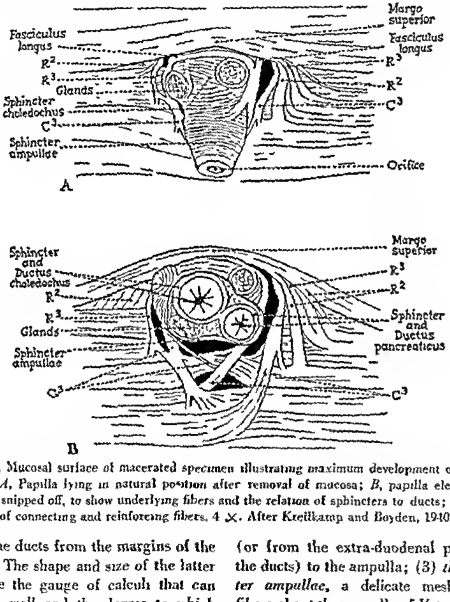

Fig. 379. Mucosal surface of macerated specimen illustrating maximum development of sphincter ampullae. *A*, Papilla lying in natural position after removal of mucosa; *B*, papilla elevated, with distal half snipped off, to show underlying fibers and the relation of sphincters to ducts; C^3 and R^2, R^3, bands of connecting and reinforcing fibers. 4 ×. After Kreilkamp and Boyden, 1940.

ing fasting), the second to shorten the intramural portion of the ducts (thus facilitating the flow into the duodenum) and the third, when strongly developed, to create abnormally a continuous channel between bile and pancreatic ducts (thus permitting reflux of pancreatic juice into the biliary tract and vice versa).

Embryologically, the sphincter of Oddi differentiates *in situ* from mesenchyme and is not an emanation from intestinal muscle, although it may connect with it subsequently. It first appears about the 45 mm. stage, as an iris-shaped ring within the eye-shaped fenestra of the circular muscle of the duodenum; then it differentiates in the direction of the papilla, much as the musculus proprius of the ureter grows from the bladder toward the kidney. Upon this gradient is superimposed a preampullary zone of growth which carries the developing sphincter choledochus away from the intestinal muscle and sets it up as an independent mechanism.

Histophysiologic Remarks. The most important part of the musculus proprius is the sphincter choledochus. During fasting this muscle retains the bile against the secretory pressure of the liver, causing it to back up into the gallbladder, the mucosa of which then concentrates it. Upon ingestion of food, the sphincter relaxes and the gallbladder contracts, with the result that concentrated bile usually reaches the duodenum within seven to fifteen minutes.

Fig. 380. Head of the hepatic duct of a 16 mm. human embryo. *B cap*, Bile capillaries; *End*, lining cells of the sinusoids; *Hc*, hepatic cells; *Hd*, hepatic duct; *Mes*, mesenchyme; *Pbc*, primitive blood cells; *Sin*, sinusoids. Note continuity of hepatic duct and its lumen with hepatic cell cords and bile capillaries respectively. Eppinger stain for bile capillaries. 700 ×.

Following cholecystectomy or chronic obstruction of the cystic duct, the sphincter of Oddi usually hypertrophies. Under other conditions it may become spastic for longer or shorter periods, often causing pain in the right hypochondrium similar to that of gallbladder disease—a condition known as *biliary dyskinesia*. Reflux

of pancreatic juice into the biliary passages, following spasm of the sphincter ampullae, is thought to be one cause of cholecystitis. In pregnancy, increase in tone of the sphincter delays the discharge of bile after meals; and in the presence of certain foods to which the patient has become sensitized, it may prevent the flow of bile entirely. In the presence of peptic ulcer, on the contrary, gallbladder bile is discharged more rapidly than under normal conditions. Only three substances are effective therapeutically in relaxing a spastic sphincter of Oddi—egg-yolk, nitroglycerine, and amyl nitrite. Animal experimentation indicates that section of specific nerves to the choledocho-duodenal junction retards, rather than accelerates the discharge of bile.

Histogenesis of the Liver and its Ducts. The liver arises early in the embryo as a diverticulum of the midgut. It appears as a ventral outgrowth which soon becomes hollow and lined by columnar epithelium; its cavity is continuous with that of the intestine. The hepatic diverticulum then extends into the mesenchyme of the septum transversum. In a 4 mm. embryo the liver consists of a thin stalk which is capped by a proliferating mass of liver cell cords. In a 10 mm. embryo the stalk has divided into two main branches which go to the right and left lobes of the liver. There is also a caudal diverticulum of the stalk which is the primordium of the future gallbladder and cystic duct. The liver cell cords continue to proliferate and even in embryos of 10 mm. contain bile capillaries. At these stages, the liver cords are distinctly tubular and may have five or six liver cells radiating around each lumen. In embryos of about 20 mm., with the ingrowth of connective tissue about the portal vein into the liver, interlobular bile ducts appear in this connective tissue and accompany the portal vein throughout its future ramifications.

As the connective tissue continues to extend into the liver substance along with the branches of the portal vein, the liver becomes divided into lobules. The exact mechanism by which the small liver of the newborn grows into the large organ of the adult is not known.

Blood formation begins very early in the liver and becomes so developed here that for a time it is the main blood forming organ of the embryo (p. 102). Blood formation stops in the liver at about the seventh month of fetal life, although this potency remains here for the life of the individual and not infrequently is brought into play in the course of certain diseases in extra-uterine life.

Bile capillaries form a continuous system in the youngest human embryos. At first these canalicules are continuous with the main hepatic ducts and, during the progressive embryonic development of the liver, with the finer branches of the interlobular ducts. There are two main theories as to the mode of origin of the ducts. The more probable of these is that the liver cells develop by branching from the head of the embryonic duct primordium and that, with the ingrowth of connective tissue into the liver substance, those liver cords nearest the connective tissue are transformed into ducts.

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Chapter XXI

PANCREAS

NEXT to the liver, the pancreas is the largest gland connected with the alimentary tract. It consists of an exocrine portion which elaborates certain digestive juices, and an endocrine portion whose internal secretion plays an important part in the control of the intermediate carbohydrate metabolism of the body. Unlike the liver, in which both exocrine and endocrine functions are carried on by the same cells, the exocrine and endocrine functions of the pancreas are carried on by distinctly different groups of cells.

Fig. 381. Photomicrograph of a human pancreas showing several islets and one large interlobular duct, 65 ×.

The pancreas is a pink-white organ which lies in the retroperitoneum at about the level of the second and third lumbar vertebrae; on the right it is intimately adherent to the middle portion of the duodenum and extends transversely across the body to the spleen. In the adult it measures from 20 to 25 cm. in length and varies in weight from 65 to 160 gm. The pancreas is covered by a thin layer of connective tissue which does not, however, form a definite, fibrous capsule. It is finely lobulated and the outlines of the

larger lobules can be seen with the naked eye. It is usually described as having a head, a body, and a tail. The head is slightly thicker than the rest and fills the loop formed by the middle portion of the duodenum, to which it is intimately adherent. It partially encircles this viscus and in very rare cases may surround it completely. The lower part of the head contains a groove through which the mesenteric vessels pass.

Exocrine Portion. The pancreas is a compound acinous gland whose lobules are bound together by loose connective tissue through which run blood vessels, nerves, lymphatics, and excretory ducts (Fig. 381). The acini which form the external secretion vary from rounded structures to short tubules. They consist of a *single row of pyramidal epithelial cells* resting on a delicate reticular membrane and converging toward a central lumen. The size of the lumen varies with the functional condition of the organ; thus, it is small when at rest, but during active secretion becomes distended with secreted material. Between the acinar cells are fine secretory capillaries which are connected with the central lumen.

The acinar cells show rather striking differences in the various stages of secretion. In general, the basal part of the cell when seen in the living condition is homogeneous or may show a faint longitudinal striation, due to the presence of filamentous mitochondria in it. The supranuclear portion—that is, the part between the nucleus and the lumen—is filled with a number of highly refractile granules. These are the secretion granules and vary greatly in number, depending on the stage of secretion. Occasionally, even in the living cell, fine clefts can be observed between these granules. This is probably the canalicular apparatus and if the secretion granules are stained supravitally, these canals become still more prominent. In sections stained with hematoxylin and eosin after Zenker-formol fixation, the basal parts of the acinar cells stain a dark purple while the secretory granules are a bright orange red.

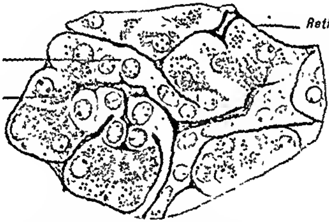

Fig. 382. Section of human pancreas showing relation of acinar to centro-acinar cells. Bielschowsky-Foot and Mallory-azan stain. 720 ×.

The *relationships between* the basophil homogeneous zone at the base (Fig. 382), apparently rich in ribose nucleoprotein, and the amount of secretory granules depend on the state of digestion; these relationships are clearly shown in the accompanying figure (Fig. 384) in which the Golgi apparatus is stained black and the secretory vacuoles appear as paler dots. In *B*, which is an acinus from a resting gland, the proximal portion of the cells is homogeneous, while the *distal portion* contains a moderate number of secretion granules, more or less separated from the

remainder of the cell by the network of the Golgi apparatus. In *A*, which is from a starving animal, the number of secretion granules has increased greatly, and in several places these have extended into the basal or proximal zone of the cell and the Golgi apparatus is much more diffuse than in *B*. In *C*, which is an acinus from a mouse which had been injected three hours previously with pilocarpine, the amount of homogeneous protoplasm is greatly increased and all of the secretory granules have been discharged into the lumen, about which the Golgi apparatus forms a limiting network.

The nucleus is spherical, it contains much chromatin, and one or two prominent oxyphil nucleoli. In some animals the cells frequently contain two nuclei, but this is relatively infrequent in man. Mitotic figures are rarely found in the acinar cells of a normal, active gland in the adult.

Between periods of active secretion, the resting cells accumulate secretory granules, apparently at the expense of the homogeneous basal cytoplasm, but, as in the case with secretion granules in general, the chemical precursors of the granules are unknown. Various theories have been set forth, it being believed by some investigators that the zymogen granules arise from the chromophil substance or from extruded nuclear material, by others from the mitochondria, and, most recently, it has been claimed that the first secretion granules during a new secretory phase arise in intimate contact with the Golgi apparatus. It has not been proved, however, that the zymogen granules arise by direct transformation from any of these cytoplasmic constituents.

Islets of Langerhans. In addition to these external secreting portions of the gland, the pancreas also contains islands of Langerhans (Figs. 381, 385). These are irregular structures, more or less completely delimited from the acini by a thin, reticular membrane and provided with a very extensive blood supply. Indeed, their great vascularity early suggested the possibility of their being endocrine organs. In some places the cells of the islands seem to be in direct continuity with either acinar cells or with undifferentiated ductule epithelium.

Fig. 383 Section of a pancreatic acinus of a guinea pig showing mitochondrial filaments embedded in the homogeneous basal substance. Secretion granules are rounded and in distal parts of the cytoplasm. Acid fuchsin methyl green stain. 1000 ×. After Bensley.

By staining the entire gland through the arterial injection of neutral red or Janus green, which stains the islands differentially (Fig. 386), it has been demonstrated that the number of islands of Langerhans in the human pancreas may vary from 208,369 to 1,760,000 in the adult organism. The number in the tail is slightly higher than in the body or head.

As seen in the usual preparations stained by hematoxylin and eosin, the islands of Langerhans seem to be composed of almost syncytium-like cords of irregularly prismatic cells which are distinctly paler than the surrounding acinar cells (Fig. 385). With such a technic, no secretion granules are to be seen in the cells of the islets. By special methods, however, it can be shown that several types of granular cells are present which

Fig. 384. Sections through three pancreatic acini of mice showing changes in the zymogen granules and Golgi apparatus: *A*, During starvation; *B*, normal pancreas; *C*, three hours after the injection of pilocarpine. *Method of Kolatschew. 950 ×. Redrawn after Nassonow.*

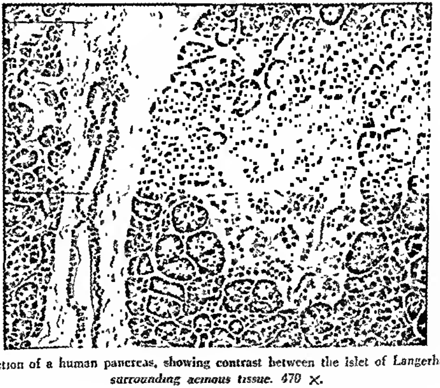

Fig. 385. Section of a human pancreas, showing contrast between the islet of Langerhans and the *surrounding acinous tissue. 470 ×.*

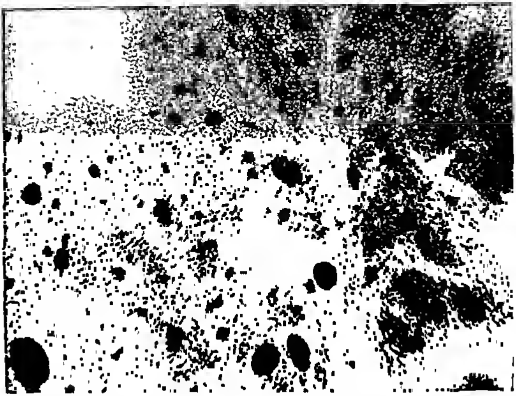

Fig. 386. Photomicrograph of a portion of the pancreas of a guinea pig, in which the islands of Langerhans have been stained differentially by the injection of neutral red into the vessels. Note the variations in size of the islands. 38 ×. After Bensley.

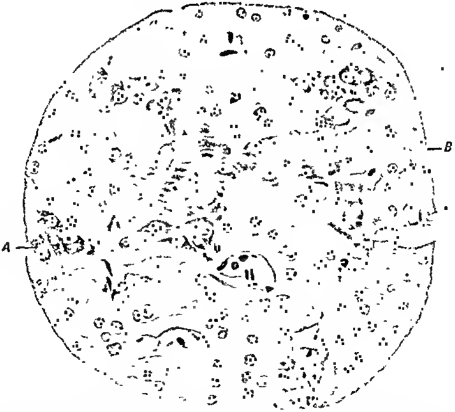

Fig. 387. Section of an island of Langerhans of pancreas of a guinea pig fixed in 70 per cent alcohol stained with neutral gentian, showing alpha cells (*A*) filled with granules. Beta cells (*B*) unstained. 600 ×. After Lane.

stain quite differently from those of the acini and accordingly must have a different constitution. One of these is found in a small number of cells, called *alpha* or *A cells* (Fig. 387, *A*); these granules are insoluble in alcohol. Other cells, the *beta* or *B cells*, constitute the bulk of the island (Fig. 387, *B*). The granules in these cells are soluble in alcohol. *In the guinea pig* some of the cells in the islands do not contain granules; these are called *C cells*.

In sections of the mammalian pancreas freshly fixed in Zenker-formol and stained with the Mallory-azan or Masson method, three types of granular cells are found in the islands (Fig. 388). The *A* cells have granules which are large and stain a brilliant red; the *B* cells have smaller brown-orange granules; while a third type of cell, called *D*, is filled with small blue stained granules (Fig. 388, *D*). Whether the *D* cells are a separate type of cell or whether they are a stage in the development of the *B* or *A* cells remains to be determined. In the dog, Hunt found the *A*, *B*, and *D* cells to constitute 20, 75, and 5 per cent of the cells, respectively.

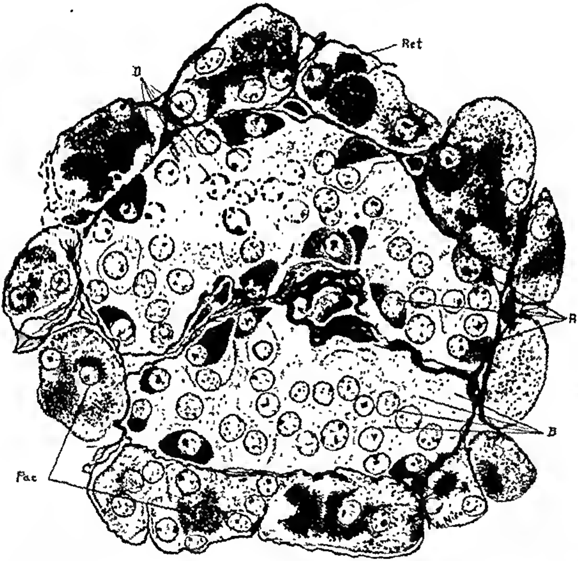

Fig. 388. Section of a human pancreas. The central part of the figure is an islet of Langerhans with granular cells of types *A*, *B*, and *D*; *Pac*, pancreatic acini; *Ret*, reticular fibers. Mallory-azan stain. 960 ×. After Bloom (1931).

Several cases of a rare disease in man have been found to be associated with small tumors of the pancreas. Most of the cells composing some of these tumors are considered to be atypical *B* cells because

of the staining reactions of their granules. Curiously, these tumor cells often contain chromophil substance like that in the acinar cells.

The mitochondria of the islands resemble those of the duct cells; that is, they are usually small rods or delicate filaments, and thus contrast sharply with the heavier, granular or filamentous mitochondria of the acinar cells. The acini contain chromophil substance and zymogen granules; the islets do not contain either of these but do have their several kinds of specific granules. The Golgi net in the islets is much smaller than in the acini.

The islands may be distributed in the following positions: (1) They may occur in the interstitial tissue, particularly along the main duct and its primary branches with which they are connected either directly by short ducts or by the system of undifferentiated tubules described below. Such islets are not connected with the acinar tissue. (2) Some islets occur intralobularly but are not connected with the acini and are connected with the intralobular system of ducts. (3) Most of the islets are located intralobularly and in direct continuity with the acini or the ducts, or both. (4) A few islets may be present in either the interstitial tissue or intralobularly, but are not connected with either the acini or the ducts.

Ducts. The pancreas usually communicates with the duodenum by a large and a small duct. The large, or main duct (of Wirsung) begins in the tail and runs through the substance of the gland, receiving throughout its course numerous accessory branches so that it gradually increases in size as it nears the duodenum. In the head of the organ, it runs parallel with the ductus choledochus with which it may have a common opening or it may open independently in the ampulla of Vater. The opening and closing of these ducts is controlled by the sphincter of Oddi (p. 437). The accessory duct (of Santorini) is about 6 cm. long. It is practically always present and lies cranial to the duct of Wirsung.

Fig. 389. Section through a small tubule near its origin from the pancreatic duct of a guinea pig showing origin of island cells from undifferentiated epithelium, *u*; *i*, island cells with granules; *c*, capillaries; *g*, goblet cells. 533 ×. Redrawn after Bensley.

The ducts represent two separate primordia. They are lined by a columnar epithelium in which goblet cells (Fig.

389) and occasional argentaffine cells are interspersed. At times small mucous glands bulge slightly from the ductal epithelium. The primary ducts are surrounded by a layer of dense, collagenous connective tissue which contains a few scattered elastic fibers. The intralobular ducts are of low columnar epithelium and rest on a reticular basement membrane. The terminals of the intralobular ducts continue into the acini as the *centroacinous cells* (Fig. 382). In sections stained with the Mallory-azan technic, the pale orange stained centro-acinous cells are in sharp contrast to the purple stained acinar cells with their bright red zymogen granules (Fig. 388).

In addition to these ducts, the pancreas contains a system of anastomosing small *tubules* which arise from the large ducts and run in the connective tissue surrounding them. These tubes have a diameter of 12 to 27 μ; they are connected with both the islets of Langerhans and with the small mucous glands and only occasionally with the acini. These structures, although studied most extensively in the guinea pig, are also said to be present in man. The epithelium of these tubules is of a low irregularly cuboidal type. They contain occasional mitoses. The cytoplasm is homogeneous in most cases. Occasional goblet cells and a few cells with true mucous granules may be found within them.

Some of the projections in these tubules consist of island cells, singly or in groups, but the most striking feature of these tubules is their connection by one or more short stalks (Fig. 390), with large islands of Langerhans. These ductules are composed of undifferentiated epithelium and it is from them that new islands, and probably also new acini, arise from time to time, particularly after injury to the pancreas. They do not carry any secretion.

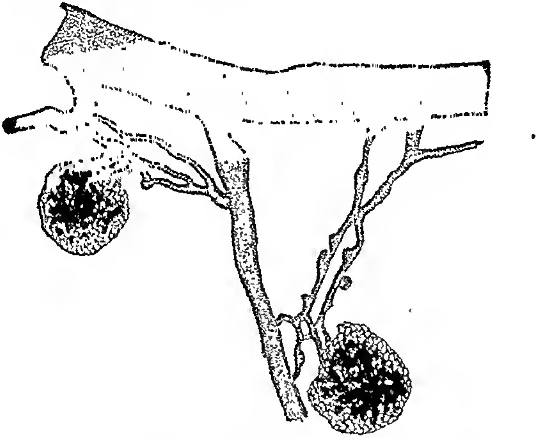

Fig. 390. Duct from the pancreas of a guinea pig showing multiple connections with two islands. 70 ×. Redrawn after Bensley.

Blood Vessels, Lymphatics, and Nerves.

The arterial supply of the pancreas is from branches of the celiac and superior mesenteric arteries. From the celiac it receives branches through the pancreaticoduodenal and the splenic arteries; it also receives small branches from the hepatic artery. The inferior pancreaticoduodenal artery is a branch of the superior mesenteric. The vessels run in the interlobular connective tissue and give off fine branches which enter the lobules Veins accompany the arteries throughout and lead the blood either directly into the portal vein or indirectly through the splenic vein.

The exact lymphatic supply of the gland has not been worked out in detail. The lymphatic drainage is mainly into the celiac nodes about the celiac artery.

The nerve supply is mainly of unmyelinated fibers arising from the celiac plexus. These fibers accompany the arteries into the gland and end about the acini with fine terminals. There are many sympathetic ganglion cells in the interlobular connective tissue. The organ also receives myelinated fibers from the vagus nerves; it has been suggested that these are of secretory nature.

Histogenesis. The pancreas arises from two diverticula of the duodenum close to the hepatic diverticulum. The two primordia of the pancreas are known as the *ventral* and *dorsal pancreases;* these fuse, and the duct of the ventral pancreas becomes part of the main pancreatic duct. The great mass of the organ is formed by the dorsal pancreas which gives rise to the body and tail and part of the head. The duct of this primordium becomes the future accessory duct. Most of the main pancreatic duct of the adult is formed from the remainder of the duct of the dorsal primordium which fuses with the duct of the ventral primordium.

At first the primordium consists of a network of anastomosing tubules lined by a single layer of cells. These differentiate into acini, in which the characteristic secretion granules appear, and also into islands. It is said that specific granules are to be found in human embryos 31 cm. long. Although the question has not been finally settled, it is quite probable that the acini do not develop into islands, but that the latter come directly from the embryonic tubules of the duct.

Regeneration. If the great mass of the pancreas is removed experimentally the organ regenerates but slightly. If a portion of the tissue be injured by a wound, mitotic figures appear in the ductal epithelium and many new islands are formed, but few, if any, new acini develop as a result of the injury. If the main pancreatic ducts be ligated, there is at first a rapid disintegration of the pancreatic acini followed by a much slower disintegration of the original islands, but at the same time ducts begin to proliferate and give rise to many new islands and to some new acini. This process extends over a period of months and even years. One week after the ligation, in the guinea pig and rabbit, most of the acini have regenerated; after one month, there is considerable regeneration of new islands and some acini from the ducts; then, most of the acini degenerate (year and a half). After nearly three years, it is said that only the main duct is present as a blindly ending structure, that there are no acini left, and that there are quite a few new islands which have arisen by sprouting from the ducts. It seems fairly well assured that the pancreas, even in the adult, is provided with undifferentiated cells which can give rise to new acini and, to a great extent, to new islands.

Histophysiologic Remarks. ***Internal Secretion.*** In view of the extreme vascularity of the islets of Langerhans, it was suggested many years ago that these structures might have an endocrine function. It was known, moreover, that extirpation of the pancreas in animals results in severe diabetes, that is, a disturbance in the carbohydrate metabolism of such a nature that the concentration of glucose in the blood rises and the excess is excreted in the urine. Such a condition results shortly in the death of the animal, but is prevented, at least in part, if the pituitary gland, as well as the pancreas, is removed.

If the pancreatic ducts are ligated the animals do not develop diabetes, although the acinar tissue degenerates, and the islands persist and may even increase in number. One of the great achievements in modern therapeutics, the insulin treatment of diabetes, rests upon the demonstration that extracts of pancreas tissue, in which degeneration of the acinar tissue had been induced by ligation of the ducts, relieved the symptoms of diabetes. Insulin was subsequently obtained from the whole or intact pancreas by preventing the destructive effect of the acinar secretion upon the internal secretion of the islets of Langerhans. Crystalline insulin is a sim-

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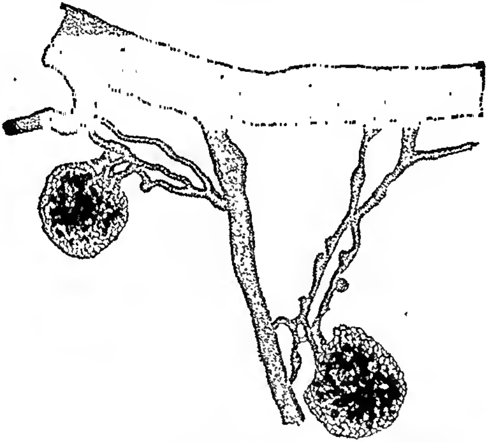

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Histogenesis. The pancreas arises from two diverticula of the duodenum close to the hepatic diverticulum. The two primordia of the pancreas are known as the *ventral* and *dorsal pancreases*; these fuse, and the duct of the ventral pancreas becomes part of the main pancreatic duct. The great mass of the organ is formed by the dorsal pancreas which gives rise to the body and tail and part of the head. The duct of this primordium becomes the future accessory duct. Most of the main pancreatic duct of the adult is formed from the remainder of the duct of the dorsal primordium which fuses with the duct of the ventral primordium.

At first the primordium consists of a network of anastomosing tubules lined by a single layer of cells. These differentiate into acini, in which the characteristic secretion granules appear, and also into islands. It is said that specific granules are to be found in human embryos 31 cm. long. Although the question has not been finally settled, it is quite probable that the acini do not develop into islands, but that the latter come directly from the embryonic tubules of the duct.

Regeneration. If the great mass of the pancreas is removed experimentally the organ regenerates but slightly. If a portion of the tissue be injured by a wound, mitotic figures appear in the ductal epithelium and many new islands are formed, but few, if any, new acini develop as a result of the injury. If the main pancreatic ducts be ligated, there is at first a rapid disintegration of the pancreatic acini followed by a much slower disintegration of the original islands, but at the same time ducts begin to proliferate and give rise to many new islands and to some new acini. This process extends over a period of months and even years. One week after the ligation, in the guinea pig and rabbit, most of the acini have regenerated; after one month, there is considerable regeneration of new islands and some acini from the ducts; then, most of the acini degenerate (year and a half). After nearly three years, it is said that only the main duct is present as a blindly ending structure, that there are no acini left, and that there are quite a few new islands which have arisen by sprouting from the ducts. It seems fairly well assured that the pancreas, even in the adult, is provided with undifferentiated cells which can give rise to new acini and, to a great extent, to new islands.

Histophysiologic Remarks. ***Internal Secretion.*** In view of the extreme vascularity of the islets of Langerhans, it was suggested many years ago that these structures might have an endocrine function. It was known, moreover, that extirpation of the pancreas in animals results in severe diabetes, that is, a disturbance in the carbohydrate metabolism of such a nature that the concentration of glucose in the blood rises and the excess is excreted in the urine. Such a condition results shortly in the death of the animal, but is prevented, at least in part, if the pituitary gland, as well as the pancreas, is removed.

If the pancreatic ducts are ligated the animals do not develop diabetes, although the acinar tissue degenerates, and the islands persist and may even increase in number. One of the great achievements in modern therapeutics, the insulin treatment of diabetes, rests upon the demonstration that extracts of pancreas tissue, in which degeneration of the acinar tissue had been induced by ligation of the ducts, relieved the symptoms of diabetes. Insulin was subsequently obtained from the whole or intact pancreas by preventing the destructive effect of the acinar secretion upon the internal secretion of the islets of Langerhans. Crystalline insulin is a sim-

ple protein with a high labile sulfur content and a molecular weight of about 35,000. All crystalline insulin so far obtained has contained zinc, and this metal is sometimes added to preparations of insulin to enhance their activity. A combination of insulin with protamines is used to extend the activity of the preparation over longer periods of time. There are many indications that the *B* cells are concerned with the production of insulin, although the proof of this is not certain.

When glucose is administered continuously intravenously in guinea pigs the drome may include cases of pituitary diabetes and even of thyroid or adrenal cortical diabetes, since all of these endocrine glands are also concerned with the regulation of carbohydrate metabolism, transport or storage. Diabetes results when there is either an absolute or a relative lack of insulin, consequently this condition may be associated with the finding of normal amounts of insulin in the organism. Dunn, Sheehan and McLetchie found that animals injected with alloxan develop a severe hyperglycemia and die; this is due to degeneration of the *B* cells.

Fig. 391. Photomicrograph of vascular injection of pancreas of guinea pig showing blood supply to an islet of Langerhans. From a preparation of R. R. Bensley. 95 ×.

B cells at first lose their granules and later become more numerous, through mitosis and through transformation of acinar cells, and loaded with granules. If the injection of glucose is continued for several days, many of the *B* cells lose their granules and become vacuolated (exhaustion). Cells like these also occur after removal of large parts of the pancreas.

The exact mode of action of insulin is still not known, but it is an important factor in the endocrine balance which determines the level of the blood sugar. It is possible, in view of the fact that pathological changes in the pancreas are not found regularly in diabetes, that this syn-

The diabetogenic action of alloxan can be prevented by a prophylactic injection of 1, 2 di-mercapto-propanol.

Accompanying the tumors of the pancreas composed of atypical *B* cells, there is a great decrease in the concentration of sugar in the blood. The symptoms of hypoglycemia in these cases may be relieved temporarily by administration of glucose, and several have been cured by removal of the tumors.

A lipotropic substance has been extracted from the pancreas and given the name lipocaic. Its relation to the other lipotropic substances is not clear (see Best, 1941).

External Secretion. The external secretion of the pancreas follows a rhythmical cycle, which seems to be dependent on the fact that in certain stages of digestion the acid content of the stomach on reaching the duodenum produces there a substance called *secretin.* This substance, carried to the pancreatic cells by the blood, induces them to secrete; then the alkaline secretion in the duodenum neutralizes the acid material from the stomach and inhibits the formation of secretin until new acid is brought from the stomach. The nature of secretin and how it acts upon the pancreatic cells are not known.

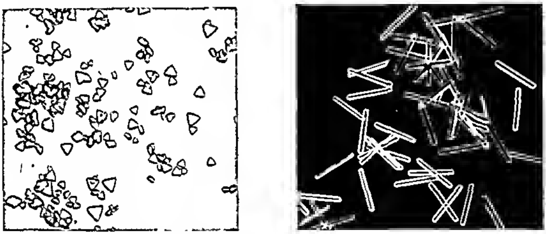

Fig. 392. Left: Crystals of trypsinogen photographed by transmitted light. 220 ×. Right: Crystals of trypsin photographed with dark-field illumination 220 ×. After Kunitz and Northrop. Courtesy of R. Northrop

The *zymogen granules* in the pancreatic acini decrease in number after the injection of pilocarpine or stimulation of the vagus nerve, and during digestion. It is claimed, however, that in normal secretion there is not an extensive diminution of zymogen granules; accordingly, the formation of pancreatic juice, rich in ferment, does not remove all of the granules. The pancreatic juice contains several types of pro-enzymes. One of these when activated becomes trypsin, a proteolytic enzyme, another an amylase or sugar-splitting enzyme, a third, lipase, a fat-splitting enzyme and, finally, an enzyme like the rennet of gastric juice.

As these enzymes are present as inactive precursors, they cannot injure the pancreas. In certain pathologic conditions, however, these pro-enzymes may be converted into the active enzymes which destroy the pancreatic tissue itself.

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Chapter XXII

THE RESPIRATORY SYSTEM

THE respiratory system serves mainly for the intake of oxygen by the body and the elimination of carbon dioxide. It may be divided into *conducting* and *respiratory portions*. The former of these comprises a series of tubelike, air-conducting structures which connect the external air with that portion of the lungs in which the exchange of gases between the blood and the air takes place. These tubes consist of the hollow passages of the nose, the pharynx, the larynx, the trachea, and bronchi of various sizes. The ends of the smallest branches of the air-conducting passages are capped by the respiratory portion of the lungs; this consists of many small air vesicles, called *alveolar sacs* and *alveoli*. The pharynx also connects the mouth with the esophagus; the larynx contains the vocal organ.

THE NOSE

The nose is a hollow organ composed of bone, cartilage, muscles, and connective tissue. Externally it is covered with skin much like that over the rest of the body and is provided with unusually large sebaceous glands and a few small hairs. The integument continues into the vestibule through the anterior nares. The epithelium here is of the stratified squamous variety and there are some fairly long hairs which are believed to help in removing particles of dust from the inspired air. The remainder of the nasal cavity is lined with ciliated epithelium and with a specialized, nonciliated epithelium in the olfactory area.

The ciliated epithelium of the nose is quite similar to that found in the larynx and trachea. It is a pseudostratified, ciliated, columnar epithelium in which goblet cells are richly interspersed. A basement membrane separates the epithelium from the underlying connective tissue layer with its mixed mucous glands. These glands secrete a mucus which keeps the walls of the nasal cavity moist. In the lower nasal conchae are rich venous plexuses which warm the air passing through the nose. These plexuses have been mistaken for erectile tissue, from which they differ, however, by the absence of septa containing smooth muscle.

The receptors for the olfactory sense are located in the *olfactory epithelium*. In fresh condition this area has a yellowish color, different from the pink color of the surrounding mucous membrane. The olfactory area extends from the middle of the roof of the nasal cavity some 8 to 10 mm. downward on both sides of the septum and on the surface of the upper nasal conchae. The total surface of these areas of both sides is estimated at about 500 sq. mm. The outlines of the olfactory area are very irregular.

The olfactory epithelium is pseudostratified columnar and about 60 μ thick. Unlike the ciliated epithelium it has no distinct basement membrane. It consists of three kinds of cells: (1) sustentacular or supporting cells, (2) basal cells and (3) olfactory cells.

The *supporting cells* are tall, slender elements with an axial bundle of tono-

fibrils. At the free surface they form very small cuticular plates which are kept together by a system of thin terminal bars. Small, round openings for the sensory cells remain between the borders of the cuticles. Under the cuticle each cell contains a diplosome, from which a tiny flagellum seems to emerge. The upper part of the cell contains a small Golgi net and pigment granules which cause the brown color of the olfactory area. The oval nuclei alternate in height in neighboring cells and thus two or three rows of nuclei are seen in a cross section.

Between the bases of the supporting cells, the *basal cells* form a single layer of small conical elements with dark nuclei and branching processes. It is uncertain whether they are reserve elements for the supporting cells.

The *olfactory cells* are evenly distributed between the sustentacular cells. They are bipolar nerve cells of fusiform shape. Their round nuclei occupy a zone in the epithelium between the nuclei of the supporting cells and the connective tissue. The peripheral part of the cell body, a modified dendrite, is thick and extends as a straight, cylindrical process from the nucleus to the surface. The proximal end rapidly tapers down into a thin, smooth filament about 1 μ thick. It is an axon—a fiber of the olfactory nerve. It passes into the subjacent connective tissue and here, together with similar fibers, forms small nerve bundles. These are collected into about 20 macroscopically visible *fila olfactoria*.

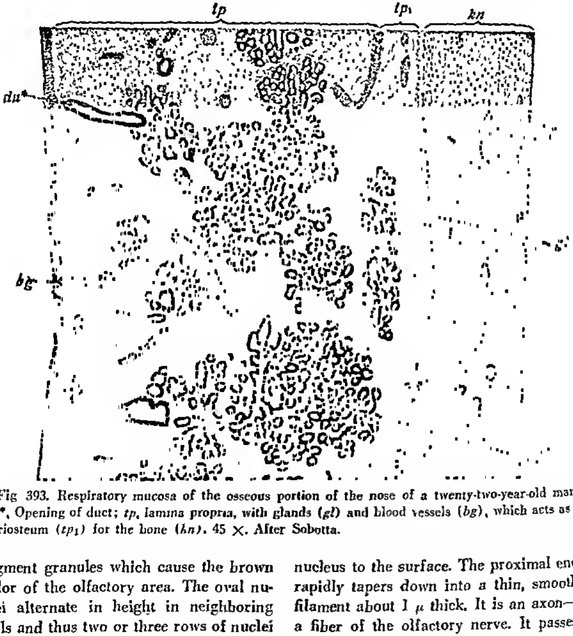

Fig 393. Respiratory mucosa of the osseous portion of the nose of a twenty-two-year-old man. *du**, Opening of duct; *tp*, lamina propria, with glands (*gl*) and blood vessels (*bg*), which acts as a periosteum (*tp₁*) for the bone (*kn*). 45 ×. After Sobotta.

The cytoplasm of the olfactory cell contains a network of neurofibrils which

are especially distinct around the nucleus; above the latter is a Golgi net. The head of the olfactory cell protrudes freely through the opening of the cuticular membrane. It enlarges slightly to the so-called *olfactory vesicle* which contains at its surface 6 to 8 tiny granules similar to the basal bodies of the ciliated cells; each

olfactory epithelium and end with fine arborizations under its free surface between the sustentacular cells. These endings are receptors for stimuli other than odors.

The *lamina propria* of the olfactory mucous membrane is fused with the periosteum. It is penetrated by the bundles of the olfactory nerve fibers. Among its cells are numerous pigment cells, and some lymphoid cells which migrate into the epithelium.

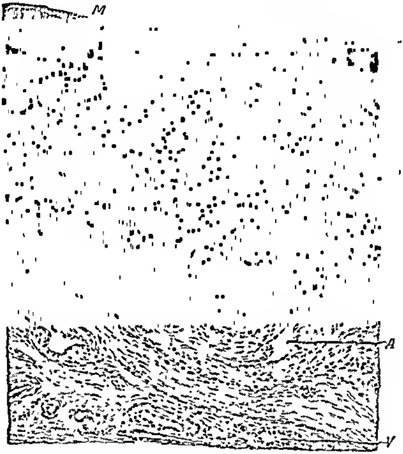

Fig. 394. Cross section of the olfactory mucous membrane on the medial surface of the middle concha, from a man: *A*, Artery; *B*, glands of Bowman; *E*, olfactory epithelium; *M*, opening of a gland on the surface; *O*, bundle of olfactory fibers; *V*, veins. 70 ×. After Schaffer.

granule sends out a fine olfactory cilium 2 μ long.

The fibers of the olfactory nerve are nonmyelinated, but are provided with a sheath of Schwann. They are kept together by a delicate connective tissue rich in macrophages. The fila olfactoria pass through the openings of the cribriform plate of the ethmoid bone and enter the substance of the olfactory bulb of the brain where the primary olfactory center is located.

Besides the olfactory nerve fibers, the olfactory mucous membrane is provided with myelinated nerve fibers originating from the trigeminal nerve. After having lost their myelin they penetrate the

Beneath the epithelium the lamina propria contains a rich plexus of blood capillaries. In its deeper layers it contains a plexus of large veins and dense networks of lymph capillaries. The latter continue into large lymphatics which course toward the lymph nodes on the sides of the head. A colored mass injected into the subarachnoid spaces of the brain can penetrate into the lymph capillaries of the olfactory region as well as into the sheaths of the fila olfactoria. This indicates some possible pathway for infections to spread from the nasal mucous membrane to the meninges.

The lamina propria in the olfactory area contains the *olfactory glands* of Bow-

man (Fig. 394, *B*), which are different from the mixed glands of the respiratory portion of the nose. They are of the branched, tubulo-alveolar type. The secretory portions are mainly parallel to the surface, while the narrow excretory duct assumes a perpendicular course and opens on the surface. Immediately under the epithelium the duct is often considerably enlarged. The cuboidal or low pyramidal glandular cells of the secretory portions have a structure similar to serous cells; even if present in extreme dilution, may become concentrated in these structures. The continuous stream of the secretion of the olfactory glands, by removing the remains of the stimulating substances, keeps the receptors ready for new stimuli. In this respect the olfactory glands doubtless have a function similar to that of the glands connected with the taste buds.

The olfactory epithelium in man is easily affected by inflammation of the mucous membrane of the nose and is often more or less altered

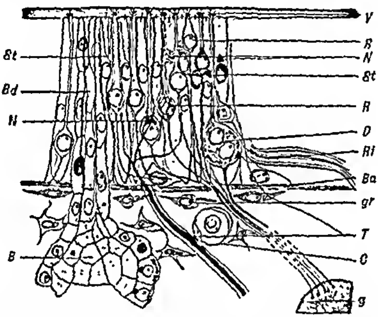

Fig. 395. Diagram of the olfactory mucous membrane of man: *B*, Gland of Bowman, with diplosomes in its cells, and with its excretory duct, *Bd*; *Ba*, layer of basal cells; *C*, capillary; *D*, large binucleated olfactory cell; *g*, olfactory glomerulus; *gr*, connective tissue cells; *N*, Golgi net in the sustentacular and in the olfactory cells; *R*, olfactory cells with neurofibrils and the olfactory vesicles, *V*; *Ri*, olfactory fibers; *St*, sustentacular cells with tonofibrils; *T*, myelinated fibers of the N. Trigeminus. After Kolmer.

they contain secretory granules. The cystic enlargements of the ducts are lined with large flattened cells which continue into the short excretory duct.

Histophysiologic Remarks. The olfactory stimuli are probably of chemical nature. The secretion of the glands of Bowman keeps the surface of the olfactory epithelium moist and furnishes the necessary solvent. As most odoriferous substances are much more soluble in lipoids than in water, and as the olfactory cells and their cilia contain a considerable amount of lipoids, odoriferous substances, and replaced by atypical epithelium.

Histogenetic Remarks. The olfactory region appears in the embryo a little later than the primordia of the eye and ear. It is found in embryos of 4.9 mm. as a paired, thickened, ectodermal area at the anterior edge of the medullary plate. Its structure at this time is similar to that of the medullary epithelium. The plate is later gradually invaginated and recedes from the surface. Some of the epithelial cells are transformed into olfactory elements, which send out axons growing toward the anterior part of the brain vesicle.

Nasal Sinuses. Connected with the *nasal cavity*, and forming cavities in the respective bones, are the frontal, eth-

moidal, sphenoidal, and maxillary sinuses —the *accessory sinuses of the nose*. They are lined with an epithelium similar to that of the nasal cavity, but containing fewer and smaller glands. The mucosa of all of the sinuses is very delicate and cannot be differentiated as a separate layer from the periosteum of the bones, to which it is usually tightly adherent.

Leukocytes and lymphocytes migrating through the epithelium, and collections of lymphatic tissue beneath it, are characteristic of the respiratory epithelium of the nose, especially near the nasopharynx.

After leaving the nasal cavity, the inspired air passes by way of the nasopharynx and pharynx to the larynx. The nasal part of the pharynx is lined by ciliated columnar epithelium. In its oral part, however, it is lined by stratified squamous epithelium which is continuous with that of the mouth above and the esophagus below. The structure of the pharynx is described on p. 371.

The connections of the lacrimal gland and the eustachian tube with the nasal cavity are discussed in the sections devoted to the eye and ear, respectively.

THE LARYNX

The larynx is an elongated, irregularly tubelike structure, whose walls contain hyaline and elastic cartilages, connective tissue, striated muscles, and a mucous membrane with glands. It serves to connect the pharynx with the trachea. As a result of changes in its shape resulting from the contraction of its muscles, it produces variations in the opening between the vocal cords. The size of this opening conditions the pitch of the sounds made by the passage of air through the larynx.

The main framework of the larynx is made of several cartilages. Of these the thyroid and cricoid cartilages and the epiglottis are unpaired while the arytenoid, corniculate, and cuneiform are paired. The thyroid and cricoid and the lower parts of the arytenoids are hyaline cartilages. The *extrinsic* muscles of the larynx connect it with surrounding muscles and ligaments and facilitate deglutition. The *intrinsic* muscles connect the cartilages of the larynx; by their contraction they give different shapes to the laryngeal cavity and thus are active in phonation.

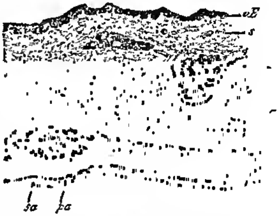

Fig. 396. Cross section through the middle of the epiglottis of a twenty-two-year-old man: *vE*, Stratified squamous epithelium of the lingual and, *hE*, of the tracheal surfaces; *K*, elastic cartilage with its perichondrium, *p*; *s*, mucosa of the lingual surface with papillae; *s'*, papilla free mucosa of the laryngeal surface; *sa*, mucous glands; *pa*, serous glands; *dr*, mixed glands. 27 ×. After v. Ebner, from Schaffer.

The anterior surface of the epiglottis and the upper half of its posterior surface, the aryepiglottic folds, and the vocal cords are covered with stratified squamous epithelium. In the adult, the ciliated epithelium usually begins at the base of the epiglottis and extends down the larynx, trachea, and bronchi.

The cilia are 3.5 to 5 μ long and beat toward the mouth; thus they move foreign particles, bacteria, and mucus from the lungs toward the exterior of the body. After death the cilia have been seen to beat for fifty to seventy hours; in tissue cultures of rabbit lung they may beat for twelve days or more.

A centriole has not been demonstrated in the ciliated epithelium of the respira-

man (Fig. 394, *B*), which are different *from the mixed glands of the respiratory* portion of the nose. They are of the branched, tubulo-alveolar type. The secretory portions are mainly parallel to the surface, while the narrow excretory duct *assumes a perpendicular course and opens* on the surface. Immediately under the epithelium the duct is often considerably enlarged. The cuboidal or low pyramidal glandular cells of the secretory portions have a structure similar to serous cells; even if present in extreme dilution, may become concentrated in these structures. The continuous stream of the secretion of the olfactory glands, by removing the remains of the stimulating substances, keeps the receptors ready for new stimuli. In this respect the olfactory glands doubtless have a function similar to that of the glands connected with the taste buds.

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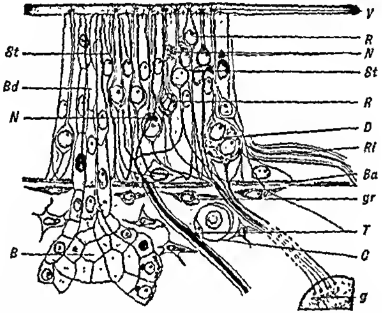

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Nasal Sinuses. Connected with the nasal cavity, and forming cavities in the respective bones, are the frontal, eth-

tion of the recurrent laryngeal nerve activates secretion in these glands. The lamina propria also contains accumulations of lymphatic tissue.

The most characteristic part of the trachea is its framework of 16 to 20 hyaline cartilages. These are C or Y-shaped and encircle the tube except in its posterior part. Because of the spaces between them they give the tube much more pliability and extensibility than if they formed a continuous sheet. The cartilages pass obliquely down the trachea. With advancing age, they become fibrous, but do not ossify as the thyroid cartilage does. They are surrounded by dense connective tissue which contains many elastic and reticular fibers.

The posterior wall of the trachea, close to the esophagus, is devoid of cartilages. Their place is taken by a thick layer of smooth muscle bundles which run mainly transversely. They are inserted into the dense, elastic fiber bundles surrounding the trachea and especially its cartilages, and are joined to the mucous membrane by a layer of loose connective tissue, some adipose tissue, and mucous glands (Fig. 399).

A delicate network of lymphatics is found in the mucosa and a much coarser plexus occurs in the submucosa. These lead into the lymphatic nodes which accompany the trachea along its entire length. The arteries for the trachea are mainly from the inferior thyroid. The nerves supplying the trachea arise from the recurrent branch of the vagus nerve and from the sympathetic. The sympathetic nerves of the trachea contain very small ganglia, from which fibers lead to the muscle of the organ. Myelinated sensory nerves are also found.

THE LUNGS*

The lungs constitute a paired organ occupying a great part of the thoracic cavity and constantly changing in form with the different phases of respiration. The right lung consists of three lobes and the left lung of two, and each lobe receives a branch of the primary bronchi. The outer surface of the lungs is closely invested by a serous membrane called the *visceral pleura.*

* This section has been revised by C. G. Loosli.

In children the lungs, because of their great blood supply, are a pale pink. With advancing age they become gray, due to the inhalation of carbon particles, particularly in city dwellers.

Fig. 398. Cross section through part of a human trachea. 60 ×. After Braus.

Each of the five lobes of the lungs is divided by thin connective tissue septa into great numbers of roughly pyramidal portions of pulmonary tissue, the lobules. These are so arranged that the apex of each points toward the hilus and the base toward the pleura. In the adult lung, these gross lobules are not so easily seen, except under the pleura, as in the embryonic lung. Under the pleura, however, the progressive deposition of carbon from the inspired air marks the outlines of these lobules very distinctly. Each lobule is supplied by a small bronchiole.

The Bronchial Tubes. The trachea divides into two main branches called

tory tract. Evidences of regeneration of the tracheal epithelium are very rare; this may be associated with the absence of a centriole. Goblet cells in varying numbers are scattered between the cylindrical

Fig. 397. Frontal section through the middle of the glottis of a boy of nine years: *K*, Thyroid cartilage with its perichondrium, *P*, *SM*, laryngeal ventricle; *St*, vocal cord; *T*, false vocal cord; *dr*, mixed mucous glands with an excretory duct, *a*; *ad*, adenoid tissue, *ce*, ciliated epithelium (pale); *m*, vocal muscle; *pe*, stratified squamous epithelium (dark); *s*, portion of the vocal cord with papillae. 15 ×. After von Ebner, from Schaffer.

cells. The glands of the larynx are of the tubulo-acinous, mixed mucous variety (Fig. 397, *dr*, *a*). Some of the ducts secrete mucus; the alveoli secrete mucus and may have crescents. A few taste buds are scattered on the under surface of the epiglottis.

The *true vocal cords* (Fig. 397, *St*) enclose the vocal or inferior thyro-arytenoid ligaments. Each of these (one on each side) consists of a band of elastic tissue which is bordered on its lateral side by the thyro-arytenoid muscle (Fig. 397, *m*) and is covered medially by a thin mucous membrane with a stratified squamous epithelium. The space between the vocal cords is usually given as 23 mm. long in men, and 18 mm. in women. Its shape undergoes great variations in the phases of respiration and in the production of different sounds in talking and singing. The thyro-arytenoid muscle is prominently concerned in phonation; on contraction it approximates the arytenoid and thyroid cartilages, and this relaxes the vocal cords.

The larynx is supplied by the upper, middle, and lower laryngeal arteries, which, in turn, arise from the superior and inferior thyroid arteries. The veins from the larynx empty into the thyroid veins. The larynx contains several rich plexuses of lymphatics which lead into the upper cervical lymph nodes and to those about the trachea. The superior laryngeal nerve sends sensory nerves, and the inferior laryngeal, motor nerves to the larynx.

TRACHEA

The trachea is a thin-walled, fairly rigid tube about 11 cm. long and 2 to 2.5 cm. in diameter. It is continuous with the larynx above and ends by dividing into the two main bronchi below.

The epithelium of the trachea is of the ciliated pseudostratified columnar type (Fig. 27), and rests on a distinct basement membrane. Numerous goblet cells are scattered throughout the epithelium. The lamina propria contains many elastic fibers and numerous small glands like those of the larynx. These glands, most of which are external to the elastic fibers, open by short ducts on the free surface of the epithelium. In the posterior portion of the trachea, the glands extend through the muscular layer (see below). Stimula-

are replaced by irregularly shaped cartilage plates which completely surround the bronchus. As a result, the intrapulmonary bronchi and their branches are cylindrical and not flattened on one side like the trachea and the extrapulmonary portions of the bronchi. At the same time as the cartilage plates become irregularly distributed around the tube, the muscular layer completely surrounds the bronchus. The cartilages disappear when the diameter of the bronchiole reaches 1 mm.

The innermost layer of the bronchi is a mucous membrane which is continuous with that of the trachea, and is lined by epithelium. The lamina propria consists of a small amount of reticular and collagenous connective tissue and many elastic fibers; it contains a few lymphoid wandering cells and is delimited from the epithelium by a basement membrane. The mucosa of the bronchi, in histologic sections, shows a marked longitudinal folding due to the contraction of the muscle. It is claimed that these folds disappear when the lung is distended.

Next to the mucosa is a layer of smooth muscles which run in all directions around the tube, but never form a closed ring as in the blood vessels and intestines (Fig. 400. *M*). The muscles form an interlacing feltwork whose meshes become larger in the smaller bronchioles. Numerous elastic fibers are intimately associated with the smooth muscle cells. As will be discussed below, the elastic fibers and smooth muscles throughout the lung play an important part in the changes in its structure

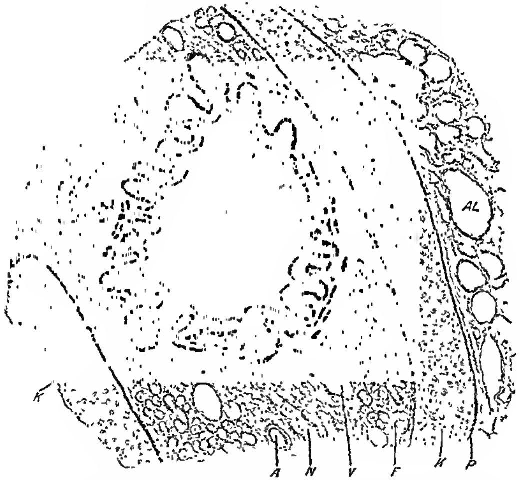


Fig. 400. Cross section through a small bronchus of a man: *A*, Artery; *AL*, alveoli; *B*, bronchus; *D*, mixed glands, *E*, ciliated epithelium with goblet cells; *F*, fat tissue; *K*, cartilage; *M*, circular muscle; *N*, nerve; *P*, perichondrium; *V*, vein. Mallory's connective tissue stain. 30 ×. After Schaffer.

bronchi. These tubes enter the substance of the lungs at the hilus, one on each side and, maintaining a downward and outward direction, divide into two smaller bronchi on the left side and three on the right. These give rise to smaller bronchi, from which *bronchioles* of several orders originate. With the development of lung 50 to 80 terminal bronchioles in each lobule. This number is probably somewhat high. Each *terminal bronchiole* continues into one, two, or more respiratory bronchioles. These break up into 2 to 11 *alveolar ducts*, from which arise the *alveolar sac* and *alveoli*. Thus, the main successive divisions of the bronchial tree are:

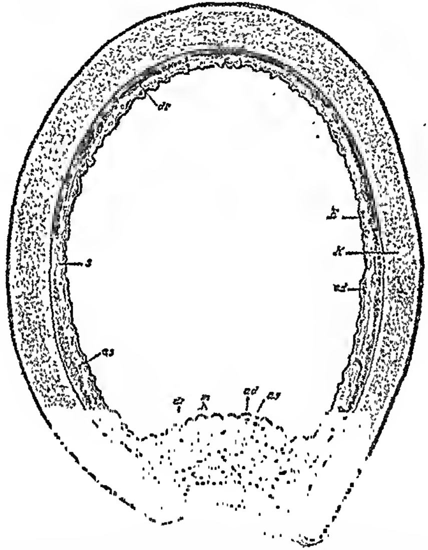


Fig. 399. Cross section through the trachea of a boy of nine years: *E*, Ciliated epithelium; *K*, cartilage; *ad*, lymphoid tissue; *dr*, glands with a duct, *as*; *m*, smooth muscle of the membranous portion of the wall; *s*, mucosa. 8 ×. After Kölliker-v. Ebner.

surgery, knowledge of the segmental distribution of the secondary bronchi in the lobes has become of importance. According to Boyden the right lung is made up of ten principal bronchopulmonary segments while the left lung can be divided into eight segments. The basic pattern of the secondary bronchi appears, however, to be subject to considerable variation. It has been estimated that there are from primary bronchi, secondary bronchi, bronchioles, terminal bronchioles, respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. An *atrium* has been described as connecting the alveolar sacs and the alveolar ducts (see p. 467).

Before the bronchi enter the lung their structure is practically identical with that of the trachea. But as soon as they enter the lung, the cartilage rings disappear and

tended with air, thick sections should be used; indeed, the structure of this organ is often seen best in sections of 60 to 120 μ. Hematoxylin and eosin staining gives but a poor idea of the constitution of the lung; special staining and injection methods are necessary. Further, the lung changes its form continuously with every inspiration and expiration (Fig. 416).

Respiratory Bronchioles. In the adult the respiratory bronchioles begin with a diameter of about 0.5 mm. They are relatively short tubes, lined in their runs. These alveoli are the first of the respiratory structures of the lung and are responsible for the term "respiratory bronchiole." These bronchioles soon branch and radiate conelike into 2 to 11 alveolar ducts which extend for relatively long distances. They are surrounded by alveoli which have arisen from adjacent ducts.

Alveolar Ducts. The structure of the alveolar ducts is hard to visualize in thin

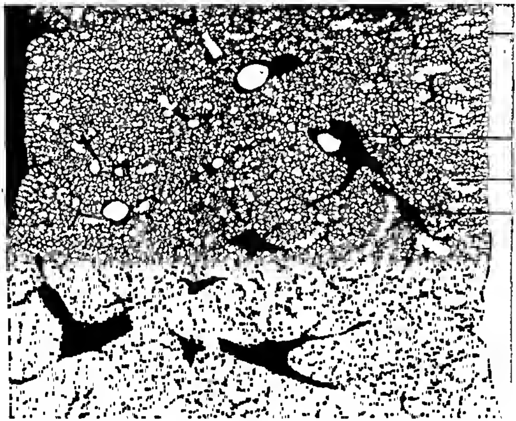


Fig. 402. Photomicrograph of a thick (120 μ) section of lung of *Macacus rhesus*. 10 ×.

first part with a ciliated columnar epithelium which contains no goblet cells A short distance down the bronchiole, the ciliated columnar epithelium loses its cilia and becomes low cuboidal (Fig. 404, *cu*). These bronchioles have walls composed of collagenous connective tissue in which bundles of interlacing smooth muscles and elastic fibers course. They lack cartilage. A few alveoli bud off from the side of the respiratory bronchiole opposite that along which the branch of the pulmonary artery

sections of the distended lung. In thick sections, particularly when studied with the binocular microscope, the alveolar ducts are seen as thin-walled tubes. They usually follow a long, tortuous course and give off several branches which in turn may branch again (Fig. 404, 405 lower). They are closely beset with thin-walled outpouchings, the alveolar sacs (and single alveoli). These are blind, polyhedral sacs which are open only on that surface which faces the alveolar duct. As the

which occur during respiration. A dense network of blood vessels accompanies and penetrates this myoelastic layer.

The outermost layer of the bronchial wall consists of dense connective tissue which contains many elastic fibers. It surrounds the plates of cartilage and continues into the connective tissue of the surrounding pulmonary tissue and into that accompanying the large vessels.

Mucous and mucoserous glands are found, as in the trachea, as far out in the bronchial tree as the cartilage extends (Fig. 400, *D*). The glands are usually

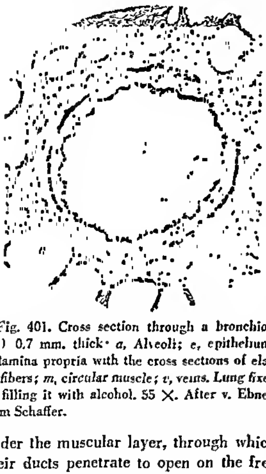


Fig. 401. Cross section through a bronchiole (*B*) 0.7 mm. thick. *a*, Alveoli; *e*, epithelium; *s*, lamina propria with the cross sections of elastic fibers; *m*, circular muscle; *v*, veins. Lung fixed by filling it with alcohol. 55 ×. After v. Ebner, from Schaffer.

under the muscular layer, through which their ducts penetrate to open on the free surface.

Lymphatic tissue, diffuse and often with nodules, occurs regularly in the mucosa and in the fibrous tissue around the cartilage, especially where the bronchi branch.

With the progressive decrease in the size of the bronchi and bronchioles as they proceed from the trachea, the layers of their walls become thinner and some of them fuse into one layer. The smooth muscle (Fig. 401, *m*), however, is distinct up to the end of the respiratory bronchioles and even continues in the walls of the alveolar ducts.

RESPIRATORY STRUCTURES OF THE LUNGS

The *unit* of the lung is composed of all the structures beginning with a respiratory bronchiole and extending to and including the alveoli with all the blood vessels, lymphatics, nerves, and connective tissue. In the newborn, the pulmonary lobule (unit) is small. The respiratory bronchiole has not yet developed and the alveoli are represented as shallow pouches on the walls of the alveolar ducts. (Compare Figures 405, above, and 414, below, with the lower part of Figure 405 and with 404.)

In a thin section of a lung the respiratory portion of the organ appears as a lacework of large spaces separated from one another by thin-walled septa (Fig. 404). Here and there, this lacework is traversed by the thick-walled bronchi and various sized arteries and veins. But quite a different picture is seen in a thick section with the binocular microscope. Here the lung appears as an irregular honeycomb in which the polyhedral alveoli and alveolar sacs form the "cells" (Figs. 402, 403). These form a honeycomb traversed by the system of bronchioles and the alveolar ducts into which the atria, alveoli, and alveolar sacs open (Figs. 410, 411).

The contradictory views on the detailed structure of the lung are due in part to several factors. The lungs must be fixed, for histologic purposes, either by way of the trachea or by injection through the pulmonary artery, with the lungs still in the body to prevent overdistention. The usual method of dropping a bit of lung into fixing fluid gives highly distorted pictures, for the lung shrinks greatly when the negative pressure of the pleura is removed and the air contained in the organ prevents the penetration of the fixative. As the pulmonary parenchyma is greatly dis-

alveolar sacs are closely packed against one another, their openings form the greatest part of the wall of the alveolar duct. The wall of the alveolar duct between the mouths of the alveolar sacs consists of strands of elastic and collagenous fibers and smooth muscle cells. In thin sections of the lung, only small portions of these fibers and muscles are seen; they appear as short knobs parallel to the long axis of the alveolar duct (Fig. 401).

In thick sections it becomes evident that the short knobs seen in the thin sections are merely tangentially cut, small portions of the long connective tissue fibers and muscle bundles which interweave in three planes between the mouths of the alveolar sacs.

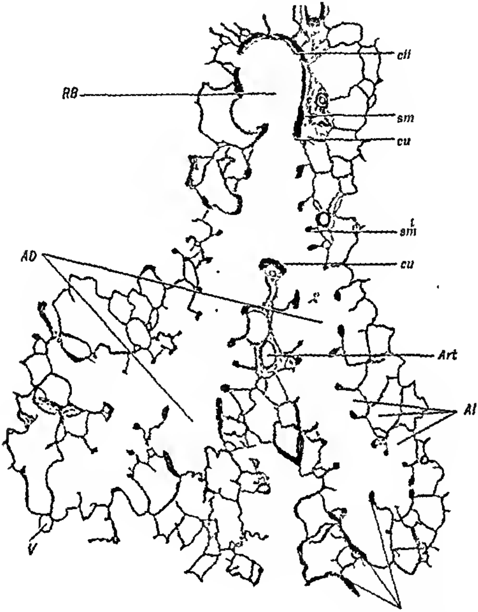


Fig. 401. Section through a respiratory bronchiole (*RB*) and two alveolar ducts (*AD*) of a human lung showing the smooth muscle, *sm* (in black), in the walls of the alveolar ducts; *cil*, ciliated epithelium; *cu*, cuboidal epithelium; *Art*, arteriole; *Al*, alveolar sacs; *V*, vein. Redrawn and slightly modified from Baltisberger.

Alveolar Sacs and Alveoli. From the alveolar ducts arise single alveoli and alveolar sacs containing two to four or more alveoli.

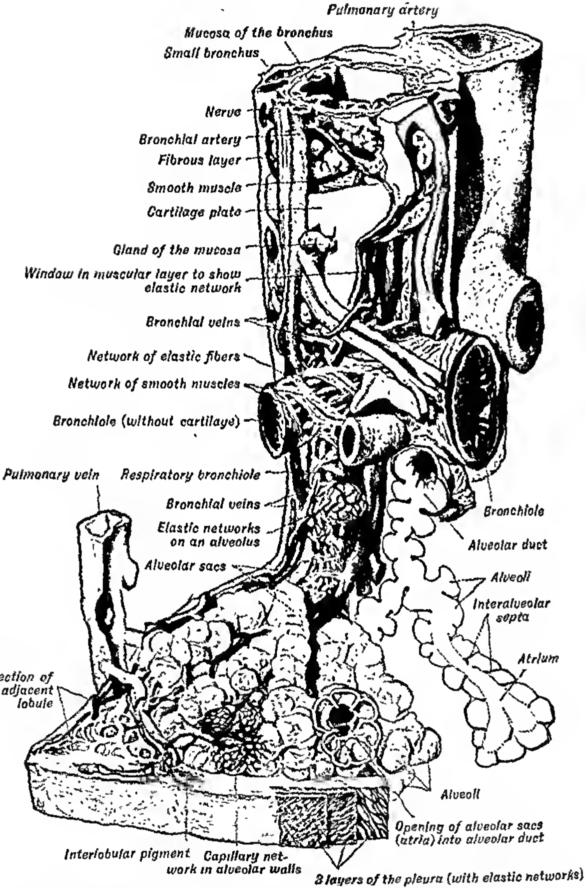


Fig. 403. Portion of a pulmonary lobule from the lung of a young man. Free reconstruction by Vierling; somewhat foreshortened. Mucosa and glands, green; cartilage, light blue; muscles and bronchial artery, orange; elastic fibers, blue-black; pulmonary artery, red; pulmonary and bronchial veins, dark blue. 32 ×. After Braus.

It has been suggested that the space between the alveolar duct and the alveolar sacs be termed the *atrium*, especially at the ends of the alveolar ducts. The structures described under this term have not been generally accepted as forming a distinct entity, for some authors consider them to be parts of the alveolar ducts.

The alveoli are thin-walled polyhedral formations, one side of which is always lacking so that air may diffuse freely from the alveolar ducts into the alveolar sacs and thus into the cavities of the alveoli. The most conspicuous feature of the alveolar walls after the age of viability is the dense, single network of capillaries in the alveolar walls. The capillaries anastomose so freely that many of the spaces between them are smaller than the diameter of the vessel lumina (Figs. 408 and 406). The alveolar walls contain a close, meshed network of branching reticular fibers. These along with a fewer number of elastic fibers form the supporting framework for the thin-walled air vesicles and their numerous capillaries (compare Figs. 410 and 411). The capillaries are so situated that the greater portion of their surface is exposed to the alveolar air (Figs. 407, 408, and 412). The larger reticular and elastic fibers occupy a central position in the septa with the *anastomosing* capillaries weaving back and forth in the meshes of the fibers to jut into the adjacent alveolar spaces. This relationship of supporting fibers to capillaries is best seen in the lung of the newborn, which has a thick cellular central stroma, and be-

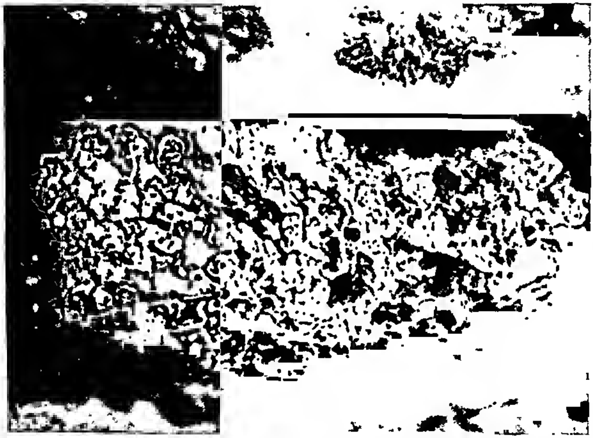


Fig. 406. Thick section of lung of eighteen-year-old man showing surface view of alveolar septum. Specimen fixed immediately after death by intra-tracheal injection of Zenker-formol solution after pulmonary veins and artery had been ligated to keep blood in capillaries. Note close network of capillaries. Mallory-azan stain. Photomicrograph. 625 ×. Courtesy of C. C. Loosli.

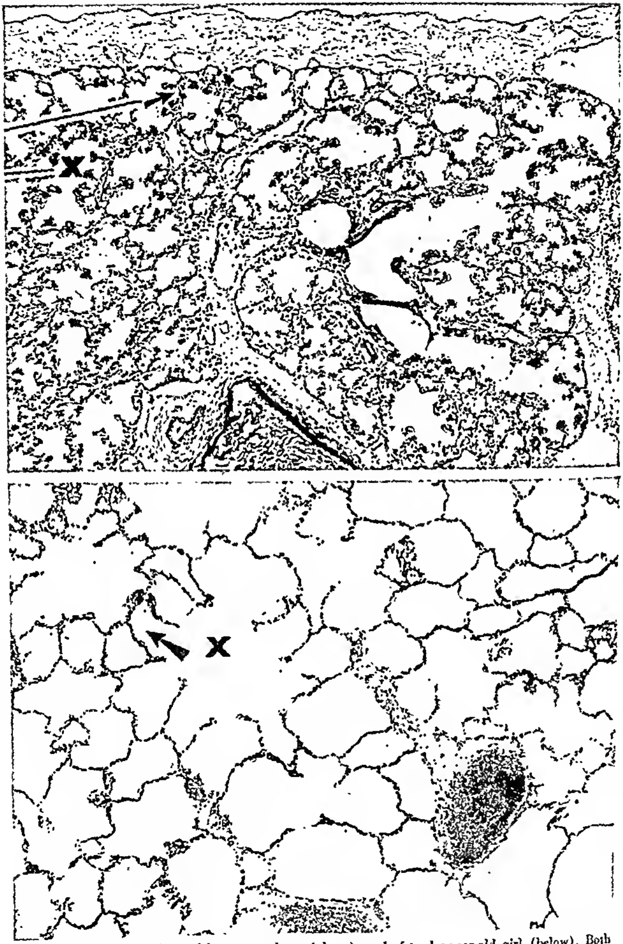


Fig. 405. Section of lung of human newborn (above), and of twelve-year-old girl (below). Both specimens fixed immediately *after death* by the intratracheal injection of Zenker-formol solution. Note increase in size of alveolar ducts (x) and alveoli (arrow head) Mallory-azan stain. Photomicrographs. 82 ×. Courtesy of C. G Loosli.

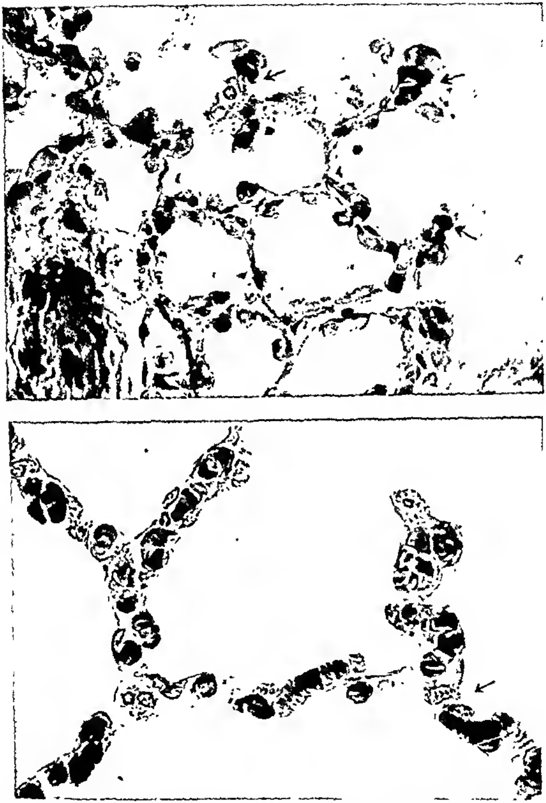


Fig. 408. Sections of lungs of newborn (above) and of a twelve-year-old girl (below), fixed as described in Fig. 405. The alveolar walls consist of a central stroma of connective tissue fibers and cells which support a close network of capillaries free of an epithelial covering. Arrowheads in the upper figure point to bundle fibers (cut in cross section) which surround the mouths of the alveoli. The arrow in the lower figure points to a septal cell. Mallory-azan stain. Photomicrographs, 600 ×. Courtesy of C. G. Loosli.

comes less conspicuous with advancing age due to the thinning and stretching of the alveolar walls.

The mouths of the alveolar sacs are completely surrounded by a wavy wreath of collagenous fibers. These continue from one sac to the next and help to give thickness to the wall of the alveolar duct (Figs. 404 and 408). It is quite probable that these curled wreaths may straighten out with deep inspirations. Elastic fibers accompany the collagenous fibers. The dense networks of reticular fibers within the walls of the alveoli and alveolar sacs are continuations of these collagenous fibers, which, in turn, are connected with the collagenous fibers in the walls of the arteries, veins, and bronchioles. The elastic fibers are likewise continuations of those of the bronchioles.

Cells Lining the Alveoli. All authors accept the presence of nucleated cells located in some of the intercapillary spaces on the alveolar walls. Some histologists, employing the intratracheal injection of silver nitrate as a method for marking the limits between cells, describe a continuous membrane composed of large, exceedingly thin plates devoid of nuclei and smaller nucleated cells. The non-nucleated plates have never been seen desquamated from the surface of the alveoli, and stages in their formation have not been described adequately. Although the question is by no means settled, there is a growing tendency to deny the existence of these plates as many of the older histologists did (see Macklin).

Fig. 407. Photomicrograph of alveolar wall of lung of eighteen-year-old man. From same specimen as Fig 406. Note the thin membrane between the lumina of the capillaries and the air spaces. Arrowheads point to "septal cells" in their characteristic location on the alveolar walls. Hematoxylin-eosin-azure II stain. 1000 ×. Courtesy of C. G. Loosli.

According to Loosli, the irregular wavy lines produced by silver nitrate introduced intratracheally or intravascularly in the alveolar walls of mammalian lungs are the outlines of the endothelial cells lining the capillary blood vessels. This is also true of the respiratory portion of the lungs of birds (see Bargmann). In frogs and turtles, the injection of silver nitrate shows that, in addition to the endothelial

association with the connective tissue stroma at the interstices of the alveolar walls (Fig. 407). Some are seen to lie across the septa with their cytoplasm projecting into the adjacent alveolar spaces. When the lung of a rabbit is completely collapsed for several weeks by repeated injections of air into the pleural cavity following section of the phrenic nerve of the same side, the nucleated cells do not thicken to form a membrane over the capillaries. On the other hand, they appear to blend with the central stroma of the walls leaving the capillaries filled with blood cells free of an epithelial covering and abutting directly against the thin clefts representing the former air spaces (Loosli). In the lungs of the newborn unexpanded by extra uterine respirations, the alveolar capillaries are likewise bare (Fig. 415).

The walls of the alveoli in the adult lung seem to consist primarily of a very dense network of anastomosing capillaries, accompanied by scattered isolated perivascular cells. Their origin from entoderm or mesenchyme or both has not been settled. The capillaries and their perivascular cells are contained in an exceedingly thin-walled, ground membrane in which the supporting reticular and elastic fibers run (Figs. 407, 408 and 412). See Macklin (1937) for an exposition of the contradictory views on this problem.

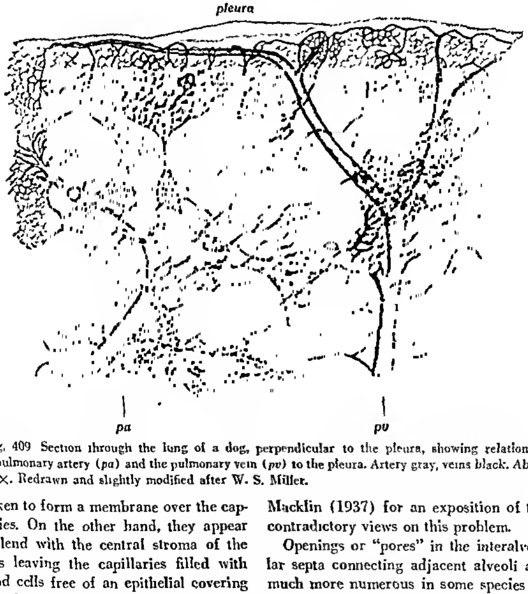


Fig. 409 Section through the lung of a dog, perpendicular to the pleura, showing relation of the pulmonary artery (*pa*) and the pulmonary vein (*pv*) to the pleura. Artery gray, veins black. About 100 ×. Redrawn and slightly modified after W. S. Miller.

Openings or "pores" in the interalveolar septa connecting adjacent alveoli are much more numerous in some species of mammals than in others. There is no doubt of their normal occurrence. In certain pathologic conditions, as lobar pneumonia, threads of fibrin can easily be demonstrated to pass through the alveolar walls and connect the inflammatory exudate in adjacent alveoli. The pores permit

cells, the alveoli are lined by a continuous layer of flattened epithelial cells.

It is obvious that the crude silver nitrate technic has classed several types of cells into one group, in which practically all cytologic details are obscured. There can be little doubt that the so-called "nucleated alveolar epithelial cells" of the older authors are composed of certain pericapillary cells (to be discussed shortly), probably also the endothelial cells of the capillaries and even some of the blood corpuscles within these vessels.

Other workers denying the existence of non-nucleated plaques consider that the alveolar surfaces are covered by thin cytoplasmic expansions of the nucleated cells, although such a membrane has not been demonstrated in normal lungs. For support of this theory, histological findings in certain chronic lung diseases showing so-called alveoli lined by a layer of cuboidal cells are described (see Miller and Bell). The walls of such spaces also show marked thickening of the connective tissue stroma and a decrease in the number of capillaries, thus resembling in no respect a normal functioning septum. The pathogenesis of such lesions has not yet been worked out. Some pathologists consider these lining cells a downgrowth of epithelium from the terminal bronchioles, secondary to destruction of the normal architecture of the alveolar walls by the disease process. Evidence to support this view is found in studies of the pathogenesis of certain experimental virus infections in animals (Loosli, Dungal) and so-called "alveolar cell tumors" in man (Herbut).

In certain strains of mice, non-metastasizing pulmonary tumors undisputedly arising from the nucleated cells, "alveolar epithelial cells," on the alveolar walls have been produced by the subcutaneous injection of carcinogenic agents. On serial transplantation in the subcutaneous tissue some of these tumors undergo change from an epithelial pattern to a fibrosarcoma (see Grady and Stewart).

In tissue cultures of the lungs and in certain *in vivo* experiments, these inconspicuous cells in the septa mobilize in a few hours and assume the appearance and function of typical macrophages. The name "septal cells" has been suggested for them (Lang). According to Loosli, in acute pneumococcal infections of the lungs of dogs and monkeys, the principal reaction of the "septal cells" appeared to be one of enlargement without detachment from the alveolar walls. No phagocytic properties were observed in the attached cells. The chief source of the macrophages was from the hypertrophy and transformation of the hematogenous lymphocytes and monocytes after they entered the air spaces in the early stages of the disease.

In practically every section of lung, free macrophages (alveolar phagocytes) can be found in the alveoli. They are indistinguishable from the macrophages in other parts of the body. When they contain particles of dust, they are called "*dust cells.*" In certain cardiac diseases they become filled with granules of hemosiderin and are then called "heart failure" cells. They are derived according to most authors from the "alveolar epithelium" or the "septal cells" as indicated above. The majority probably arise from the agranulocytes of the blood which have wandered into the alveoli and hypertrophied into macrophages. Whatever the embryologic origin of the cell, it certainly acts in the defense of the lung, including the removal of the dust particles, as a typical macrophage (see Robertson, 1941).

The nature of the nucleated cells, "septal cells," outside the capillaries in the alveolar walls is not clear. While some speak of them as being epithelial, others regard them as of connective tissue origin. In the normal lung, they are in intimate

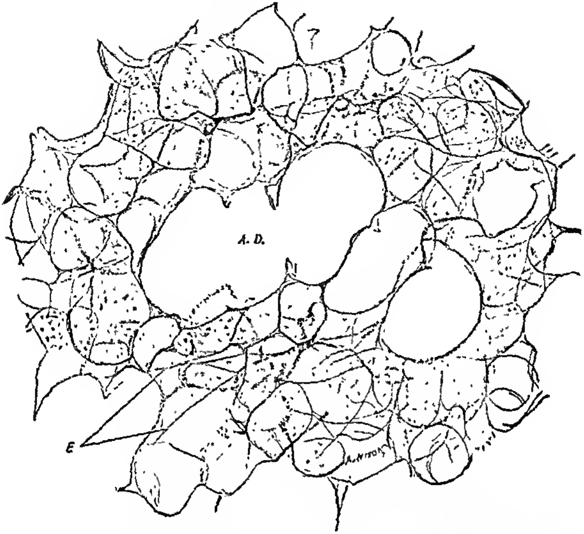


Fig. 411 Portion of the lung of a monkey (*Macacus rhesus*), fixed *in situ* and stained with resorcin-fuchsin for elastic fibers and counterstained with light green; *A. D.*, Alveolar duct; *E*, elastic fibers. Very thick section. Compare with Fig. 410.

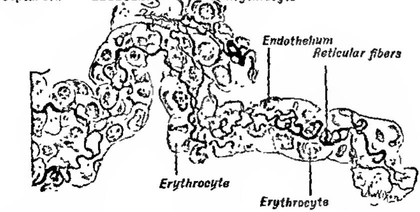


Fig. 412. Section of an interalveolar septum of a human lung. The erythrocytes seem to be separated from the air spaces only by the endothelium of the capillaries. Bielschowsky-Foot and Mallory-azan stains. 720 ×.

the spread of bacteria from one alveolus to its neighbors in pneumonia. They also provide a collateral air circulation, which aids in preventing atelectasis when secondary bronchi become obstructed.

Blood Vessels. The lungs receive most of their blood from the pulmonary arteries. These vessels are of large caliber and of the elastic type (p. 238). The branches of these arteries in general accompany the bronchi and their branches as far as the respiratory bronchioles. The arterial paths in the lung, however, are subject to considerable variation. It would appear that the rather easily resectable bronchopulmonary segment should not be considered to be a morphologic bronchovascular unit (Boyden). From the respiratory bronchioles they divide and a branch passes to each alveolar duct and is distributed in a capillary network over all the alveoli which communicate with this duct (Fig. 409). The venules arise from the capillaries of the pleura and from the capillaries of the alveolar septa and portions of the alveolar ducts and run in the intersegmental connective tissue, independently of the arteries, and fuse to form the pulmonary veins. In passing through the lung, the pulmonary artery is usually above and behind its accompanying bronchial tube while the vein is below and in front of it.

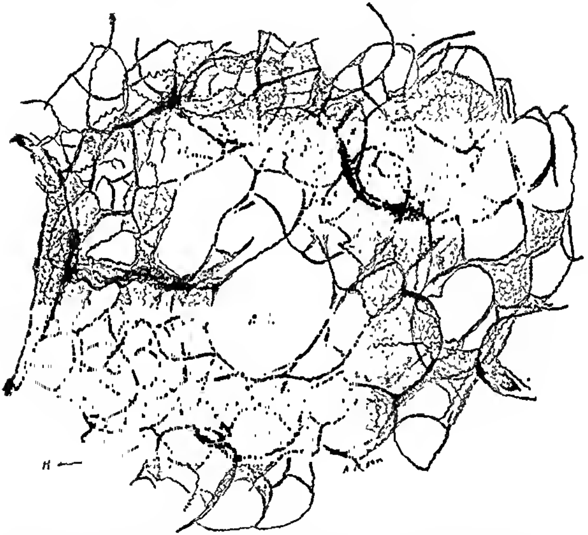


Fig. 410. Thick section from the same block as Fig. 402, stained for reticular fibers (*R*) by the Bielschowsky-Foot method: *A. D.*, Alveolar duct. Note how much more numerous are the reticular fibers than the elastic fibers in Fig. 411.

The bronchial arteries and veins are much smaller than the pulmonary vessels. These arteries arise from the aorta or the intercostal arteries and follow the bronchi. They are distributed to the walls of the bronchi, their glands, and the interlobular connective tissue beneath the pleura. Most of the blood carried by the bronchial arteries is brought back by the pulmonary veins. In the alveoli which arise from the respiratory bronchioles, there is a capillary anastomosis between the terminations of both the pulmonary and bronchial arteries.

Lymphatics. There are two main divisions of

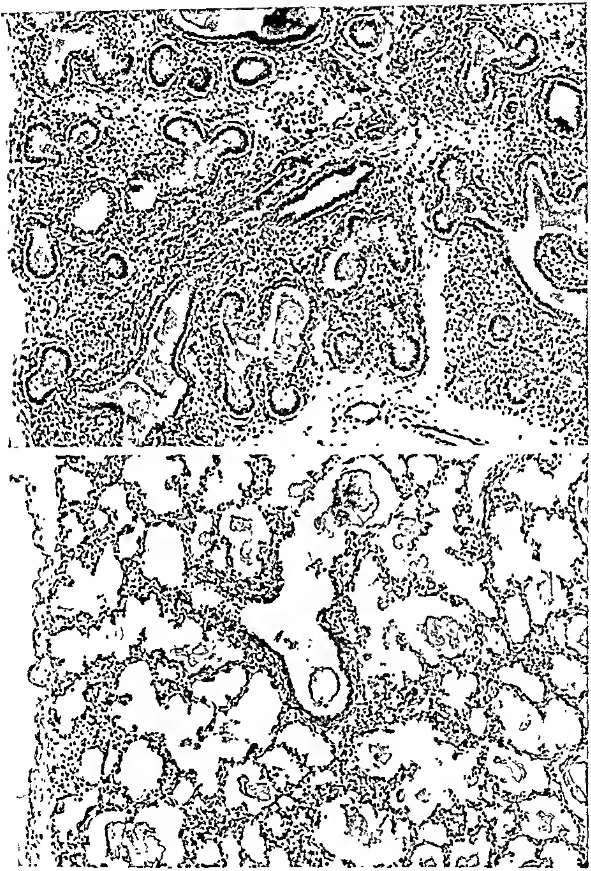


Fig. 414. Photomicrographs of section of lung of 147 gm. (4 months) fetus, above, and of a 440 gm. (6½ months) fetus, below. Both specimens show thorotrast aspirated by intrauterine respirations. The lung of the 6½ months fetus was expanded by extrauterine respiration. Note change in character of lung structure from glandular type (previable) to respiratory type (viable). Respiratory portion of lung of older fetus consists essentially of alveolar ducts; alveoli are absent or are represented only by shallow indentations on duct walls. Hematoxylin and eosin stain. 45 ×. After Davis and Potter.

the lymphatics of the lungs. One set is in the pleura and the other in the pulmonary tissue; they communicate very infrequently; both of them drain into the lymph nodes at the hilum of the lung. The lymphatics of the pleura form a dense network with large and small polygonal meshes. The large meshes are surrounded by large vessels and demark the lobules; the small meshwork is formed of smaller vessels which mark out the anatomical unit. There are many valves in these lymphatics which control the flow of lymph so that it passes to the hilum and not into the pulmonary tissue. These pleural lymphatics combine into several main trunks which drain into the lymph nodes at the hilum.

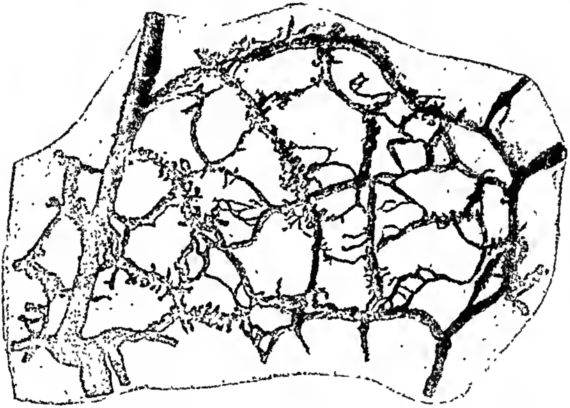


Fig. 413. Injected lymphatic plexus of the pleura of a human lung. The area bounded by the large vessels is the base of a pulmonary lobule. It is composed of numerous, smaller, polygonal areas demarcated by the smaller lymphatics; these correspond to the pulmonary units. 8 ×. After W. S. Miller.

The pulmonary lymphatics may be divided into several groups which include those of the bronchi, of the pulmonary artery, and of the pulmonary vein. The lymphatics in the bronchi form an anastomosing network. They terminate in the alveolar ducts and their end branches join the lymphatic radicles of the plexuses about the pulmonary artery and vein. There are no lymphatic vessels beyond the alveolar ducts. The pulmonary artery is accompanied and drained by two or three main lymphatic trunks. The lymphatics associated with the pulmonary vein begin with its radicles in the alveolar ducts and in the pleura. All of the lymphatics of the pulmonary tissue drain toward the hilus nodes. Efferent trunks from the hilar nodes anastomose to form the right lymphatic duct which is the principal channel of lymph drainage from both the right and left lung. There are no valves in the intrapulmonic lymphatics except in a few vessels, in the interlobular connective tissue near the pleura, which accompany the branches of the pulmonary veins. These lymphatic vessels connect the pulmonary and pleural lymphatic plexuses. As their valves point only toward the pleura, they provide a mechanism whereby lymph can flow from the pulmonary tissue into the pleural lymphatics if the normal flow of lymph in the former toward the hilum is interrupted.

As was mentioned above, the mucous membrane of the bronchi is infiltrated with lymphocytes and often contains lymphatic follicles. There are other accumulations of lymphatic tissue in the adventitia of the pulmonary arteries and veins, but these, as a rule, do not form nodules in the normal lung.

Nerves. The pulmonary plexuses at the root of the lung are formed by branches of the vagus and from the thoracic sympathetic ganglia. The bronchoconstrictor fibers are from the vagus nerve, while the bronchodilator fibers are

alveolar ducts and the alveoli. Whether growth of the lung takes place only by an increase in size and distention of existing ducts and alveoli or by some other process needs further study.

Repair of the Lung. The lung is frequently the seat of *inflammatory conditions which leave* it unimpaired on healing. There are certain infections, however, in which large masses of pulmonary tissues are destroyed, notably tuberculosis. In this case healing is always attended by connective tissue scar formation; there is no evidence to show that the pulmonary tissue can regenerate after destruction.

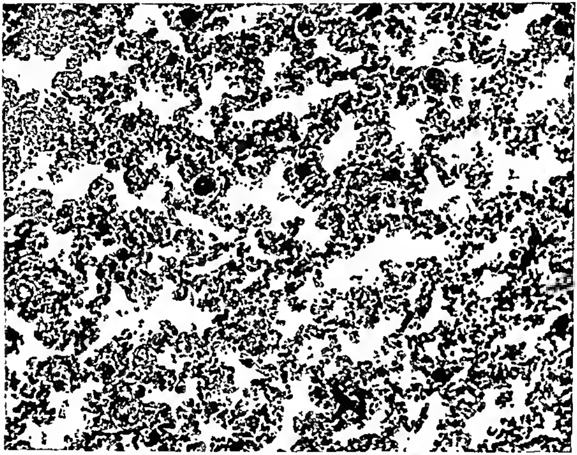


Fig. 415. Section of lung of fetus at term unexpanded by extrauterine respiration. Note marked vascularity of alveolar walls and absence of glandular character. After Adair and Potter.

Histophysiologic Remarks. The primary function of the lungs is to serve as a means for the assimilation of oxygen from the air and for the removal of carbon dioxide from the body. The network of blood capillaries in the wall of the air vesicles is separated from the air by a thin, moist membrane which permits the ready diffusion of oxygen into the blood and carbon dioxide out of it. The question as to whether the passage of the gases is to be looked upon as a secretion or as a simple process of diffusion must be decided in favor of the latter view for the present, *except* that *liberation of carbon* dioxide from carbonic acid by dehydration is now known to be greatly accelerated by an enzyme—*carbonic anhydrase.* The capillaries in the respiratory portions of the human lung are estimated to have a surface area of 140 square meters. The lung also eliminates approximately 800 cc. of water a day in the expired air; under abnormal conditions it may also remove certain substances from the blood, such as alcohol.

The lung has a large margin of reserve; that is, the body at rest uses but a small portion—about $\frac{1}{20}$—of the pulmonary aerating surface.

Recent studies indicate that the alveoli

from the sympathetic and arise mainly from the inferior cervical and first thoracic ganglia. The pulmonary vessels are supplied with both sympathetic and parasympathetic nerve fibers. Their effect on these vessels is not understood, as the experimental evidence is contradictory. The sympathetic fibers act as vasoconstrictors for the bronchial arteries.

The Pleura. The serous membrane lining the pleural cavities is reflected over the lungs as the *visceral pleura*. It consists of a thin layer of collagenous tissue containing some fibroblasts and macrophages, and several prominent layers of elastic fibers running at various angles to the outer surface. It is covered by a layer of mesothelial cells like those of the peritoneum. A similar serous layer lines the wall of the thoracic cavity and is called the *parietal pleura*. A prominent feature of the pleura is the great number of blood capillaries and lymphatic vessels distributed in it. The few nerves of the parietal pleura are connected with the phrenic and intercostal nerves. The nerves to the visceral pleura are believed to be branches of the vagus and sympathetic nerves supplying the bronchi.

Histogenetic Remarks. The lung arises in the embryo as a medial diverticulum of the foregut, caudal to the branchial clefts; it extends caudally and divides into two branches. The medial diverticulum is the primordium of the future larynx and trachea, and the first two lateral branches will form the two main bronchi of the adult lung. These two branches divide repeatedly; they become surrounded by a relatively dense mass of mesenchyme, so that throughout most of the embryonic period, up to six months, the lung has a suggestively glandlike structure (Fig. 414, upper).

The primitive bronchi are lined with cuboidal epithelium. They branch dichotomously and are capped by end buds lined with cylindrical epithelium. The lumen of the end knobs is distinctly larger than that of the ducts. The knobs continue to branch perpendicularly to the axis of their ducts. In the three- to four-month stage the connective tissue cells and fibrils become prominent and the connective tissue contains many blood vessels. The lymphatics at this time are large and divide the pulmonary tissue into fairly distinct lobules. The end knobs now begin to branch irregularly.

In the eighteen- to twenty-week stage the lobulation becomes decidedly less prominent as the connective tissue diminishes in amount and the lymphatics become much narrower. The end knobs become much smaller.

At six to seven months, in a 35-cm fetus, the small bronchi are lined in part with ciliated epithelium, which flattens toward the peripheral ends of the bronchi. These branch and end in the terminal ducts capped with the end knobs. The latter finally become alveoli, according to most authors. This view is probably incorrect.

At about the beginning of the sixth month of gestation, the lung undergoes rapid structural alterations. It loses its gland-like appearance (compare Figs. 405 and 415) and becomes a highly vascular organ. The cuboidal entodermal cells lining the end buds disappear and are replaced by a network of blood capillaries which lie on the surfaces of the relatively thick-walled, saccular air spaces.

The factors which are responsible for these morphological changes are not known. Although the human fetus begins a pattern of respiratory activity early in intrauterine life (Davis and Potter), such activity is not necessary for the normal development of the lungs (Potter and Bohlender).

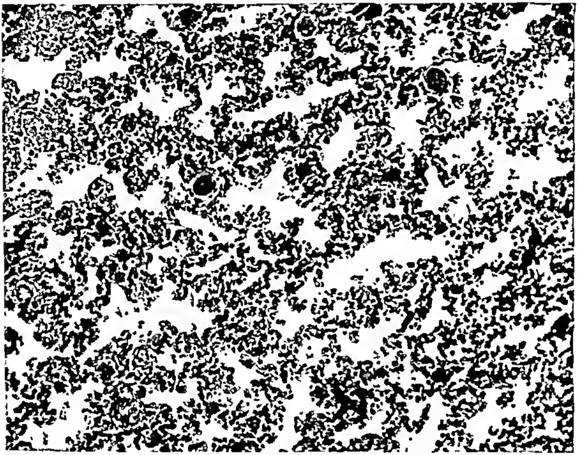
It seems clear that in human embryos (Palmer, Barnard and Day) and in pig embryos (Clements, Ham and Baldwin), the continuous epithelial membrane becomes interrupted and replaced by blood vessels on the walls of the future air spaces The fate of the cuboidal entodermal cells lining the end buds as the lung continues to grow has not been determined completely. Whether some of them persist or are replaced by cells of mesenchymal origin can only be settled by a thorough embryologic investigation of the lung in the later stages of intrauterine life.

The manner in which the lung grows beyond the initial gland-like stage is not known. In all probability, the few cells of epithelial origin which remain on the walls of the future air spaces have little if anything to do with the new formation of alveoli. More important in the postnatal growth of the respiratory portion of the lung is the further development of the elastic, smooth muscle, and vascular systems (Loosli, 1938).

The majority of investigators consider the initial respiratory air spaces to be alveoli similar in size to those seen in the adult lung (Bremer, Willson). In man it would seem that these saccular spaces correspond more correctly to alveolar ducts and that definitive alveoli are absent (Fig. 414). At term, the alveoli are shallow indentations on the respiratory channels (Fig. 405). According to Dubreuil and co-workers, the adult type of respiratory unit does not become apparent until several years after birth. One has only to compare in Fig. 405 the inflated lung of the newborn with the expanded lung of a twelve-year-old, to note the marked increase in size of the

alveolar ducts and the alveoli. Whether growth of the lung takes place only by an increase in size and distention of existing ducts and alveoli or by some other process *needs further study.*

Repair of the Lung. The lung is frequently the seat of inflammatory conditions which leave it unimpaired on healing. There are certain infections, however, in which large masses of pulmonary tissues are destroyed, notably tuberculosis. In this case healing is always attended by connective tissue scar formation; there is no evidence to show that the pulmonary tissue can regenerate after destruction.



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immediately, because the force which normally opposes the contraction of its elastic elements has been removed. This condition is known as *pneumothorax*. Such a lung remains collapsed until a negative pressure develops in the pleural cavity by the absorption of the air contained in it.

With each inspiration the descent of the diaphragm enables the bronchi in the lower lobes of the lungs to extend. And, since the main bronchi are not fixed in the thorax, but descend on inspiration, a mechanism is provided whereby the bronchi of the upper lobes of the lungs extend at the same time (see Fig. 416).

The lung of a normal adult who has lived in the vicinity of a large city is usually greatly blackened by a pigment which on chemical analysis has been found to be carbon. This material has been inhaled with the air; part of it is returned to the exterior by the action of the cilia in the bronchi; part of it is expelled in the so-called "dust cell" and part of it is accumulated in the interstitial lymphoid tissue of the lung or in the peribronchial and peritracheal lymph nodes. The particles of dust in the alveoli are taken up by the ameboid "dust cells." It is quite probable that these cells by their own motion reach the ciliated epithelium in the terminal bronchioles or enter the finer lymphatic radicles. Carbon-containing macrophages may be seen frequently in the lymphatics but the manner in which they enter these vessels has not been described.

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probably change but little during inspiration and the flow of blood is actually faster then. It is becoming more and more probable that the great increase in the volume of the lungs in inspiration takes place mainly through a great distention of the alveolar ducts. A significant finding is that the smaller bronchi and bronchioles also distend with inspiration.

reticular fibers are put under still greater tension. This is a purely passive activity on the part of the lung. In expiration, as the thoracic cavity becomes smaller, the negative pressure in the pleural cavity is lessened. This decreases the tension on the elastic and reticular fibers and they pull the lung into a more contracted state, thus forcing some of the air out of it. It is quite probable that the smooth muscles of the alveolar ducts and the bronchioles also help force the air out of the lung by their contraction.

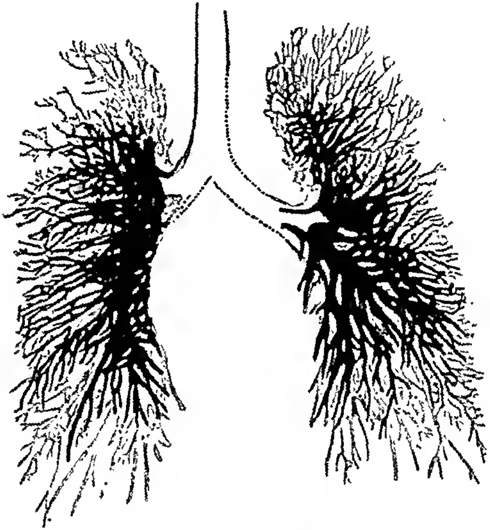

Fig. 416. Tracings from x-ray shadows of human lungs in deep inspiration (gray) and forced expiration (black). Redrawn and slightly modified after Macklin.

The pressure within the lung is that of the atmosphere. The lungs are maintained in a partially distended position by the negative pressure of the potential space between the two layers of the pleura. An increase in the size of the thorax, such as occurs with every inspiration, increases the negative pressure in the pleural cavity; consequently the lung sucks in more air and becomes larger, and its elastic and

When the pleural cavity is connected with the outside air, either by accident or by surgical intervention, the pressure in the lungs and the pleural cavity becomes equalized at that of the atmosphere. The lung in this side of the chest collapses

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Chapter XXIII

URINARY SYSTEM

THE KIDNEY

The mammalian kidney is a compound tubular gland which elaborates the urine. Its glandular tubules are called uriniferous tubules and are provided with a peculiar filtration apparatus, a tuft of capillaries, the glomerulus.

The human kidney is a paired, bean-shaped body, situated in the posterior part of the abdominal cavity, one on either side of the vertebral column. From the excavated edge, or *hilus,* a large excretory duct, the *ureter,* leads to the *urinary bladder* which is the reservoir for the urine. The *urethra,* the last section of the excretory passages of the kidneys, conveys the urine outside the body. The male urethra also serves for the discharge of semen.

The kidney is loosely invested by a *capsule* of dense collagenous bundles and a few elastic fibers. The glandular part of the kidney surrounds a large cavity, the *sinus,* adjacent to the hilus (Fig. 417). The sinus contains the *renal pelvis* and is filled with loose connective and fat tissue through which the vessels and nerves pass to the renal tissue.

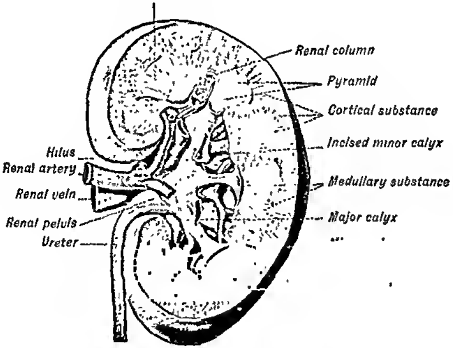

Fig. 417. Human kidney, seen from behind after the removal of a part of the organ. Three-fifths of natural size. After Braus.

The pelvis is an enlargement of the excretory passages of the kidney. Distally it is continuous with the ureter; toward the renal tissue, it forms two or more outpocketings, the *major calyces.* These again are provided with a varying number of

smaller outpocketings, the *minor calyces.*

The glandular substance consists of an outer mass—the cortex—which covers the medullary substance composed of 8 to 18 *renal pyramids* (*of Malpighi*). These are roughly conical bodies placed with the base outward and with the apex, or *papilla,* projecting into the lumen of each minor calyx. The papilla is perforated by 10 to 25 small openings—the *area cribrosa.*

guished on macroscopic examination of the fresh section.

Where the dark brown cortex separates the lateral surfaces of the pyramids it forms the *renal columns* (*of Bertin*) (Fig. 417). From the bases of the pyramids thin, radially directed processes arise which enter the cortical substance but do not reach the surface of the organ. They show the same striation as the substance

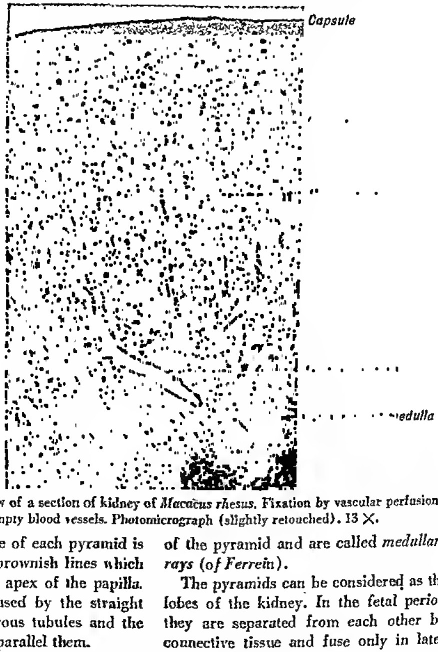

Fig. 418. Low power view of a section of kidney of *Macacus rhesus.* Fixation by vascular perfusion—hence the empty blood vessels. Photomicrograph (slightly retouched). 13 ×.

The gray substance of each pyramid is radially striated by brownish lines which converge toward the apex of the papilla. This striation is caused by the straight parts of the uriniferous tubules and the blood vessels which parallel them.

Owing to the different character of the straight tubules in its various levels, each pyramid can be subdivided into an inner and an outer zone. In the latter, again, a darker and thicker inner, and a lighter and thinner outer layer can be distin-

of the pyramid and are called *medullary rays* (*of Ferrein*).

The pyramids can be considered as the lobes of the kidney. In the fetal period they are separated from each other by connective tissue and fuse only in later stages, although in the ox, and sometimes in man, the lobated condition remains throughout life.

Each pyramid can be subdivided into smaller structural units, the *renal lobules,*

on the basis of the branching of the excretory ducts. Adjoining lobules are not separated from one another by connective tissue partitions (see p. 491).

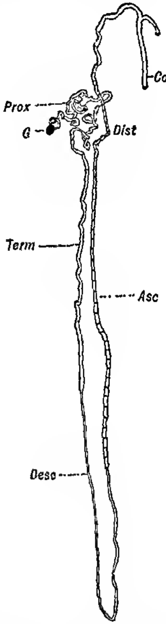

Fig. 419. Metanephric renal tubule of adult rabbit, completely isolated by teasing. *Prox*, proximal convolution; *Term*, terminal part of proximal convolution; *Desc*, descending limb of Henle's loop; *Asc*, ascending limb of Henle's loop; *Dist*, distal convolution; *Col. t*, collecting tubule; *G*, glomerulus. Slightly modified after Huber, from Cowdry's Special Cytology, published by Paul H. Hoeber, Inc.

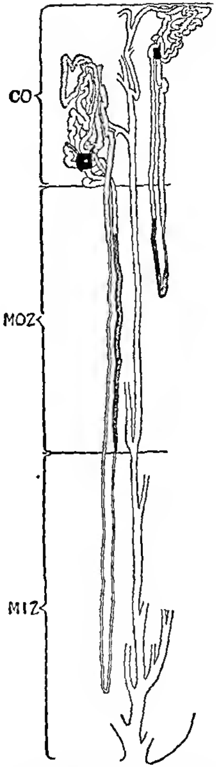

Fig. 420. Diagram of two nephrons and their connection with the collecting tubules. *CO*, Cortex; *MOZ*, outer zone of medulla; *MIZ*, inner zone of medulla; Malpighian corpuscles black; proximal convolution stippled; descending limb of Henle white; ascending limb of Henle crosshatched and then white (to indicate the opacity and clearness seen in macerated preparations but not in sections); distal convolution obliquely striated. Collecting tubules white. Redrawn and slightly modified after Peter.

Uriniferous Tubules. As in most other glands, the kidney contains two kinds of tubules: the first are the secretory portions which help form the urine,

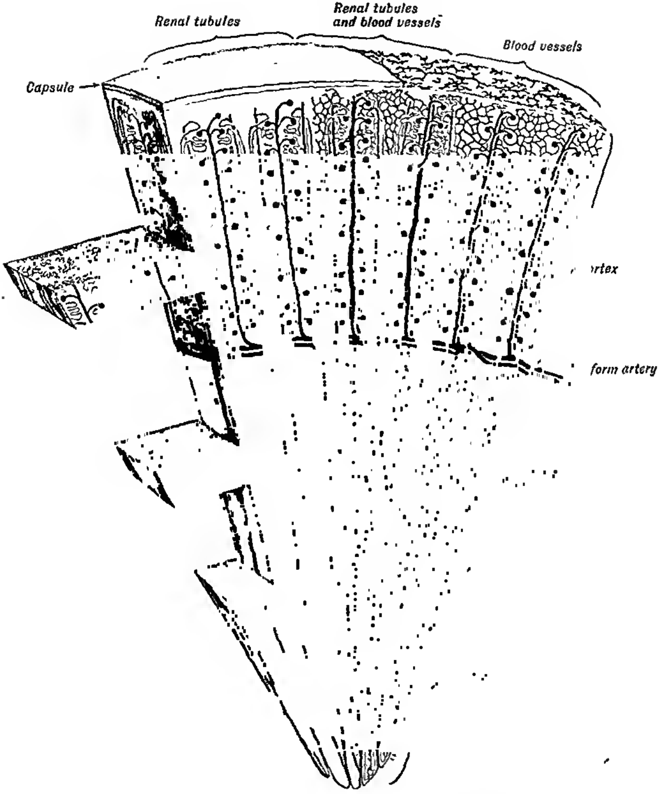

Fig. 421. Diagram of relations of blood vessels, nephrons, and collecting ducts in kidney. The actual structures are much more complicated than those indicated here. *Arteries*, red; *veins*, blue; *glomeruli*, red dots; *nephrons*, green; *collecting ducts*, black. Six interlobular arteries and attached glomeruli are shown. The right-hand pair shows their relation to the veins, the left-hand pair their relation to nephrons, and the central pair shows their relations to both nephrons and veins. Extensively modified from the diagrams of Peter, Braus, and v. Möllendorff.

while the second are excretory ducts which convey the urine to the ureter. In contrast to other glands, which arise from a single primordium, the secretory portions of the kidney tubules develop from the metanephrogenic blastema and unite secondarily with the excretory ducts which arise from the Wolffian duct. The secretory portions are long tortuous simple tubes, each of which ends in a saclike enlargement, the *capsule of Bowman*. As the uriniferous tubules follow a very tortuous course, the form and the relation of the tubules can be elucidated only by teasing the tissue after maceration or by reconstructing the tissue in wax from serial sections.

The Secretory Portion, the Nephron. Each tubular secretory portion, beginning with the capsule of Bowman and ending at the junction with the excretory ducts, is the structural and functional unit of the kidney—the *nephron* (Figs. 419, 420). Each part of the nephron has a peculiar configuration, occupies a definite position in the cortex or the medulla and is lined with a specific type of epithelium. The successive parts of the nephron are: renal corpuscle with its capsule of Bowman, the proximal convolution, descending and ascending limbs of Henle's loop, and distal convolution. A short connecting tube joins the collecting tubule or excretory duct with the nephron (Fig. 419).

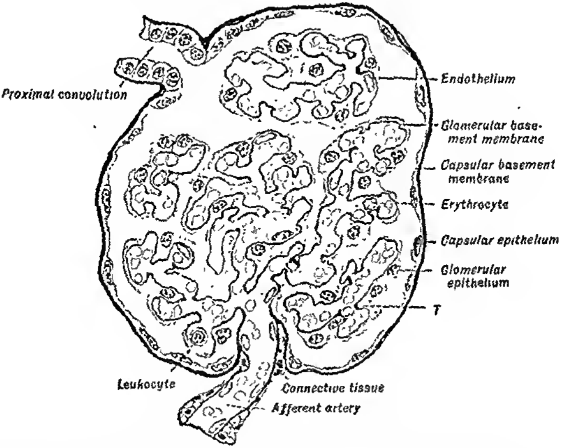

Fig. 422. Diagram of a section through the greatest diameter of a normal human glomerulus. *T*, thickening of glomerular basement membrane—often seen at orifice *into* another loop. Slightly modified after McGregor

Renal Corpuscle. The capsule of Bowman arises in the embryo as a spherical epithelial vesicle which is later invaginated by a tuft of blood capillaries, the glomerulus, and thus is transformed into a double-walled cup. The capsule and the glomerulus together form the roughly

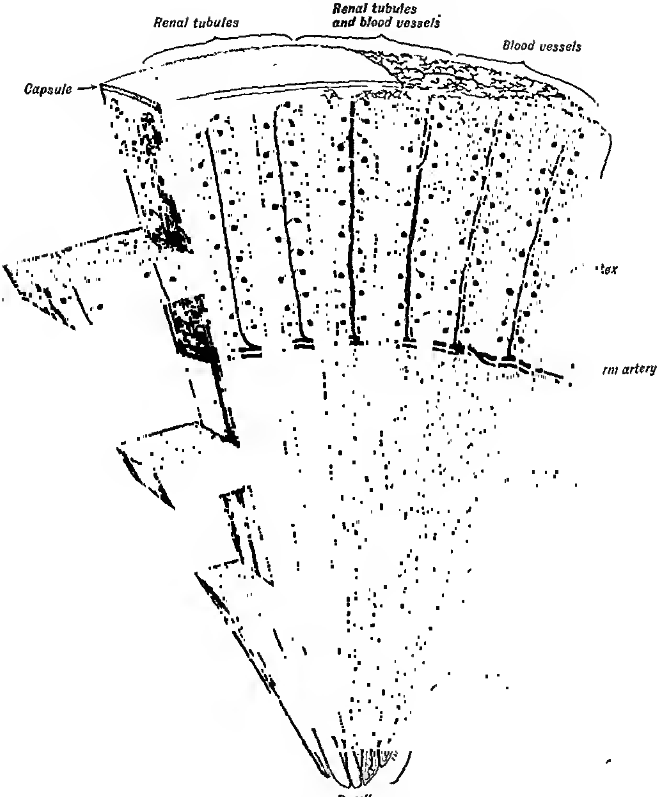

Fig. 421. Diagram of relations of blood vessels, nephrons, and collecting ducts in kidney. The actual structures are much more complicated than those indicated here. *Arteries*, red; *veins*, blue; *glomeruli*, red dots; *nephrons*, green; *collecting ducts*, black. Six interlobular arteries and attached glomeruli are shown. The right-hand pair shows their relation to the veins, the left-hand pair their relation to nephrons, and the central pair shows their relations to both nephrons and veins. Extensively modified from the diagrams of Peter, Braus, and v. Möllendorff.

the cells in the carotid body. They form a cuff extending for 25 to 85 μ along the arteriole and because of their position have been called the *juxtaglomerular cells.* They are absent from the lower vertebrates and from children below the age of two years. Unlike the cells in the carotid and aortic bodies, which have a very rich supply of nerves, the innervation of the juxtaglomerular cells is not different from that of the unchanged smooth muscle cells

the cellular outlines in the endothelium of the glomerular capillaries, so that this endothelium is supposed to have a partly syncytial character. Each capillary loop is covered by a basement membrane on which rest the visceral epithelial cells. The epithelial nuclei are believed to be ten times as numerous as the endothelial nuclei. A few reticular fibers have been found in the glomerulus and, according to some, a few fibroblasts.

Fig. 424 Section in the long axis ... *Macacus rhesus.* Photomicrograph. 58 ×.

of the vas afferens. The internal elastic lamella is well developed but ceases with the branching of the vessel.

The vas afferens, entering the renal corpuscle, divides into 2 to 4, rarely up to 10, primary branches, which in their turn divide into secondary branches. From these arise about 50 very tortuous capillary loops which only exceptionally anastomose before they fuse to form the vas efferens.

Silver nitrate fails to bring out all of

The efferent arteriole has a layer of circular smooth muscle fibers but is devoid of an elastic membrane. Its diameter is distinctly smaller than that of the afferent vessel. It is probable that the contractility of the vas efferens helps regulate the pressure in the glomerulus.

Proximal Convolution. The short transition from the capsule to the following part of the tubule is *sometimes* referred to as the "neck," although a marked constriction is by no means typi-

spherical renal corpuscle (of Malpighi). The original cavity of the capsule of Bowman is transformed into a cleftlike space. At one pole of the corpuscle the outer or "parietal" layer of the wall continues into the next section of the nephron. On the opposite side the glomerulus is connected with its afferent and efferent arterioles. Here the parietal wall is reflected onto the surface of the glomerulus as the "visceral" layer of the capsule (Fig. 422). The glomeruli are scattered throughout the cortical tissue between the medullary rays.

Estimations of the number of glomeruli vary from 1,000,000 or even less to about 4,500,000 in one human kidney. Since the glomerulus, as is explained below, is essentially a filtration apparatus, the size of its free surface must be of importance. The absolute values given by various investigators (e. g., 0.78 square meter for all the glomeruli of one kidney) cannot be considered as reliable.

The "visceral" epithelial cells covering the surface of the glomerulus adhere closely in a continuous layer to the capillary loops (Fig. 422). They contain a few mitochondria and a Golgi net. The claim that the visceral epithelium consists of isolated perivascular mesenchymal cells or pericytes has not been generally accepted. The "parietal" epithelium is usually of the simple squamous type and the limits of its polygonal cells can be demonstrated fairly well.

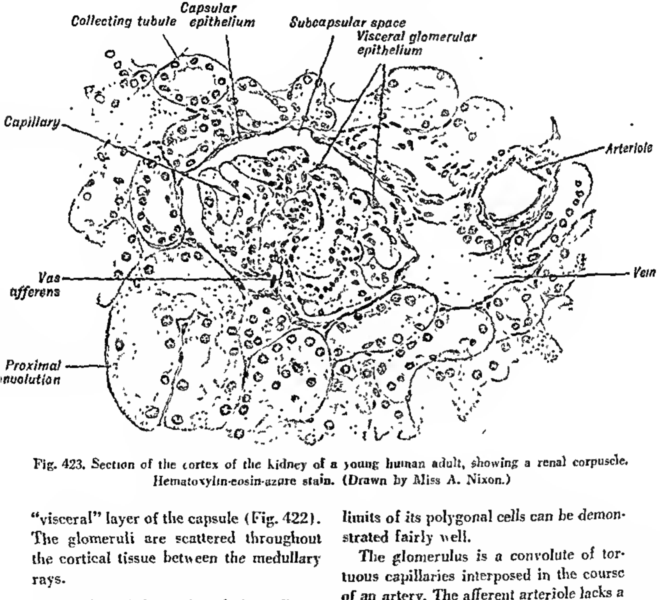

Fig. 423. Section of the cortex of the kidney of a young human adult, showing a renal corpuscle. Hematoxylin-eosin-azure stain. (Drawn by Miss A. Nixon.)

The glomerulus is a convolute of tortuous capillaries interposed in the course of an artery. The afferent arteriole lacks a distinct adventitial coat; its media consists of circularly arranged smooth muscle cells. Near the entrance of the vessel into the glomerulus, the smooth muscle cells of the vas afferens become large and pale-staining, an appearance which gave them the name epithelioid. They suggest

logic conditions, as well as in postmortem autolysis, droplets of neutral fat and of phosphatids readily appear. In some animals (cat) they are of normal occurrence.

During diuresis the lumen of the proximal convolution is large, the cells are low and flattened, and the brush border high (Fig. 425, *B*). In the resting condition the cells are high and provided with a bulging surface which transforms the lumen into an irregular, star-shaped cleft (Fig. 425, *A*). Other structural changes, as vacuolization of the apical parts, bulging of drops of the superficial cytoplasm through the brush border into the lumen, as well as the formation of vacuolated or foamy masses in the lumen all seem to be artefacts.

Although the whole length of the proximal convolution seems to have the same structure, certain experiments (vital staining, poisoning with uranium, chromium, bichloride of mercury, etc.) allow a subdivision of this tubule into three or four successive stretches, which show specific reactions to the different noxious factors.

Loop of Henle. The proximal convolution continues into the loop of Henle. This has two straight limbs running parallel to each other in the radial direction and connected by a sharp bend or crest. The descending limb of the loop is a thin, straight tubule with an outer diameter of 14 to 22 μ. The ascending limb is thicker (33 μ). The transition from the thin descending to the thick ascending limb is usually abrupt and rarely occurs at the crest of the loop.

The loops of Henle are of different lengths, depending on the level they reach in descending into the pyramid and on the position of the renal corpuscle to which they belong. In the human kidney the short ones are about seven times as numerous as the long ones; they belong to those renal corpuscles located nearer to the surface of the kidney; their crest is always formed by the thick ascending limb and is located in the outer zone of the medulla The thin, descending limb may be very short or even absent. In the latter case the proximal convolution continues directly into the thick ascending limb. In the longer loops which belong to the deeper renal corpuscles the crest is formed by a thin limb. These loops sometimes extend nearly to the apex of the papilla. In this case the length of the thin limb may vary from 4.5 to 10 mm. or even more

The ascending thick limb of the loop of Henle has, on the average, a length of 9 mm. It occupies a position nearer to the corresponding collecting tubule than the descending limb. This part of the nephron is usually located in the outer zone of the medulla.

As the proximal convolution passes into the descending thin limb of the loop, the caliber of the lumen tapers down gradually but the epithelium changes abruptly.

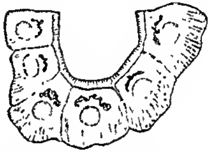

Fig. 426. Transection of a proximal convolution of guinea pig under high magnification Between nucleus and brush border the Golgi net is seen. Redrawn after Brugnatelli

The cells with the brush border are suddenly replaced by thin, squamous epithelial cells with a pale staining cytoplasm containing a slightly flattened nucleus which causes a bulging of the central part of the cell body into the lumen. The cell outlines are also sometimes distinctly festooned, *e. g.*, in the cat (Fig. 427). The edges of the free surfaces are provided with terminal bars. At the free surface, above the nucleus, a pair of centrioles with a central flagellum is present; a Golgi net has also been described; mitochondria are very scarce.

Owing to the thinness of the epithelium, the descending limbs of the loops can be easily mistaken for blood capillaries (Fig. 428).

The change from the descending limb to the ascending limb is usually also fairly

cal. The next part of the tubule is called the *proximal convolution* and is the most important functional section of the nephron. It is 14 mm. long and 59 μ in diameter, has a very tortuous course and constitutes most of the cortical substance. In addition to many small loops it always forms a large loop directed toward the periphery, then returns to the vicinity of its renal corpuscle, approaches the nearest medullary ray, and runs toward the medulla. In the outer zone of the pyramid, the proximal convolution tapers down into its terminal portion which continues into the U-shaped *loop of Henle.*

easy to preserve them satisfactorily in fixed material. After suitable fixation and staining, the basal parts of the cells are occupied by parallel rods, perpendicular to the basement membrane and reaching the level of the nucleus. They stain like mitochondria. In poorly fixed slides this typical striation of the epithelium is invisible because the rods disintegrate into granules. This also occurs during life as a degenerative process in pathologic conditions. The superficial part of the cell body contains granular mitochondria and often small vacuoles which stain supravitally with neutral red.

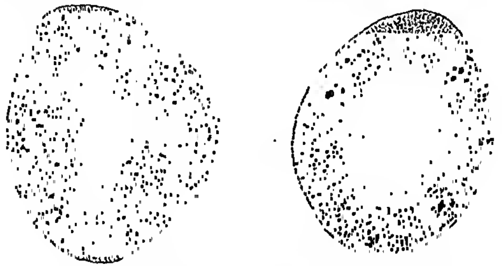
A B

Fig. 425. Cross section through the proximal convolution, showing brush border, of a dog's kidney. *A*, In anuria; *B*, at maximal secretion. After Sauer, from Metzner.

The epithelium of the proximal convolution consists of one layer of truncated pyramidal cells. Their cytoplasm is abundant and stains deeply with eosin; each cell contains a large, pale, spherical nucleus. Only three or four nuclei are usually seen in a transection of the tubule. The limits between the cells are rarely seen because the sides of the cells are provided with grooves and ridges perpendicular to the *basement membrane* and interdigitating with the same structures of the adjacent cells.

In fresh condition the cells appear opaque and granular. They undergo postmortem changes very rapidly and it is not

The free surface of the cells lining the lumen is covered by a *brush border* (Figs. 425, 426). The limit between the latter and the cytoplasm is marked by a layer of granules which in section appear as a darkly staining line. Under this line, above the nucleus, are a pair of centrioles and a Golgi net; in compensatory hypertrophy of the kidney this organoid recedes to the infranuclear region. The edges of the free surfaces of the cells, under the brush border, are provided with a system of terminal bars.

In the normal human kidney the epithelium of the proximal convolution does not contain lipoid inclusions. In patho-

collecting tubules the cells are cuboidal, sharply outlined, contain a darkly staining round nucleus and have a very clear cytoplasm. The latter contains a few fine mitochondria and, at the surface, a pair of centrioles with a central flagellum.

As the collecting tubules grow larger, the cells also grow higher, and finally acquire in the ducts of Bellini a tall columnar form. They are always arranged in a very regular, single layer, with all the nuclei at one level and with the free surfaces bulging slightly into the lumen. The cytoplasm keeps its pale appearance. The centrioles remain at the free bulging surface. In the area cribrosa the simple columnar epithelium of the ducts of Bellini continues onto the surface of the papilla.

The length of the collecting tubules is estimated at 20 to 22 mm.; the length of the nephron at 30 to 38 mm.

Renal Lobule. Each renal lobule is composed of the glandular tissue surrounding a medullary ray. The slender, pyramidal form of the renal lobule and the presence of a medullary ray forming its core are the results of the straight, radial course of the collecting tubules, of the gradual increase of their branchings toward the capsule and of the accumulation of nephrons in the cortex. Each medullary ray contains several straight collecting tubules, the loops of Henle, and the terminal portions of the proximal convolution. It is surrounded by the convoluted portions of the tubules and by the renal corpuscles. At the apex of the lobule, which reaches far into the papilla of the pyramid, the collecting tubules begin to fuse with one another and finally open, as the ducts of Bellini, on the area cribrosa. A large number of lobules form the Malpighian pyramid.

A few authors believe that the renal lobules center about the radial branches of the arciform artery extending toward the capsule of the organ. According to this view, the interlobular arteries are really *intralobular* arteries. In view of the embryonic development of the organ from a series

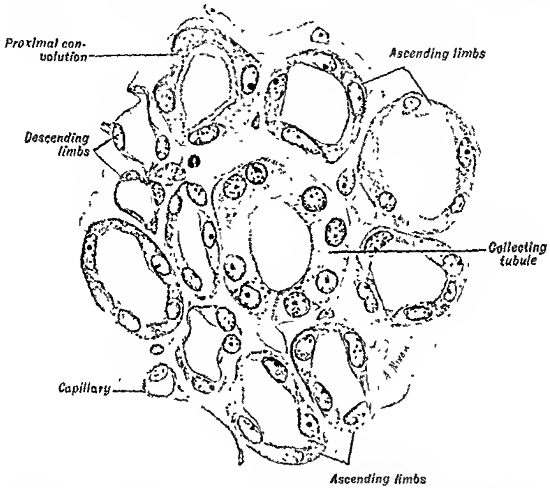

Fig. 428. Section of a medullary ray from the kidney of *Macacus rhesus.* The organ was fixed by perfusion—hence the slightly dilated condition of the tubules Iron-hematoxylin stain. About 800 ×.

sudden. The epithelium becomes cuboidal and stains darker. Rod-shaped mitochondria cause a distinct perpendicular striation of its basal part, where the cell boundaries are not clearly seen. The superficial parts of the cells, where granular mitochondria occur, are, on the contrary, sharply outlined. The nucleus is spherical or slightly flattened. A pair of centrioles occupies the free surface and seems to be provided with a central flagellum.

The ascending limb of Henle's loop enters the cortical tissue, returns to the renal corpuscle of its nephron and attaches itself to its vascular pole, particularly to the vas afferens with its juxtaglomerular cells. That side of the tubule

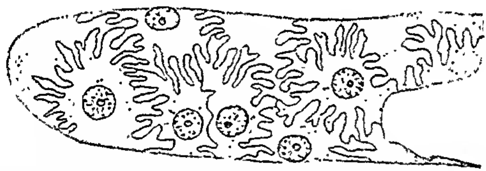

Fig. 427. Oblique section of the proximal (thin) branch of the loop of Henle; kidney of a cat. Cells with highly serrated outlines; in some of them a diplosome is seen. Iron-hematoxylin stain. 1270 ×. Redrawn after Zimmermann.

in contact with the vas afferens forms an elliptical disc of tall thin cells measuring 40 by 70 μ in man. This area is called the *macula densa*. From here the loop gradually passes into the *distal convolution.*

Distal Convolution. This portion of the tubule has many outpocketings and zigzag contortions; it usually forms a loop directed toward the periphery and above the corresponding renal corpuscle. Its length is estimated at 4.6 to 5.2 mm., its diameter at 20 to 50 μ.

In the distal convolution the epithelium is lower and the lumen is larger than in the proximal convolution. The free surfaces of the cells show irregular projections and are devoid of a brush border, but possess the usual pair of centrioles. In the basal parts of the cells a more or less distinct striation can be seen; the limits of the cells are fairly distinct. The cytoplasm stains less intensely with acid dyes than in the proximal convolution. In a cross section the cells are more numerous than in the latter; instead of 3 to 4, 5 to 8 nuclei can usually be counted. Each distal convolution continues by a short connecting tubule into one of the initial branches of the *collecting* tubules.

Excretory Ducts or Collecting Tubules. The connections of the collecting tubules with the nephrons are located in the cortex along a medullary ray and are called the "peripheral branchings" of the collecting ducts. From the medullary ray the collecting tubules pass radially inward through the outer zone of the medulla without further fusions. When they reach the inner zone, they fuse at acute angles with other similar tubules and this is repeated seven times. These fusions or branchings are in the medulla near the pelvis and are called the "central branchings (or fusions)." Through the central fusions very large, straight collecting tubules are formed, the so-called *papillary ducts* (of Bellini), with a lumen measuring 100 to 200 μ. They open on the area cribrosa on the apex of the papilla.

The system of the intrarenal excretory ducts has a very typical epithelium, quite different from that of the various parts of the nephron (Fig. 428). In the smallest

the medullary rays—the lobules of the kidney—they are called *interlobular arteries*. Those who hold that these arteries form the center of the renal lobule call them *intralobular arteries*.

The interlobular arteries give off numerous smaller branches, each of which is the afferent vessel of a glomerulus. From the glomerulus the blood is carried away by the efferent vessel. The vas efferens breaks up into a network of arterioles which supply the convoluted uriniferous tubules of the cortex with blood. It is probable that the efferent artery of a glomerulus supplies much of the nephron belonging to the same renal corpuscle.

The tissues of the medulla and the medullary rays also receive their blood supply from the efferent glomerular arterioles. The medullary rays are supplied by the efferent vessels of the nearest glomeruli. The pyramids of Malpighi are supplied by the *arteriolae rectae spuriae* which arise as efferent vessels from the glomeruli near the medulla. The arteriolae rectae spuriae assume a straight, pelvipetal course. This arrangement causes a radial striation of the outer zone of the Malpighian pyramid. After having entered the medullary substance, they branch profusely at acute angles and form a capillary nework with radially elongated meshes about the straight tubules.

The existence of the so called "arteriolae rectae verae," which are supposed to arise directly from the arcuate or the interlobular arteries and to enter the medulla, is denied by most investigators. It has been claimed that apparently direct communications between arterioles and venules may arise through partial obliteration of glomeruli.

The veins of the cortex arise from the capillary networks in the outermost layers of the cortex through the confluence of radially arranged branches, which form the so-called *stellate veins*. These continue into interlobular veins, which follow the corresponding arteries and everywhere receive additional capillaries from the labyrinth. The interlobular veins fuse to form the large arcuate or arciform veins which run beside the arteries of the same name. In the medulla straight, radially arranged veins arise (*venulae rectae*) which join the arciform veins directly. The latter fuse and form interlobar veins which accompany the large arteries and finally give rise to the renal vein.

Lymphatics. Networks of lymphatic capillaries are found in both the capsule of the kidney and in the glandular tissue. Both groups are connected by occasional anastomoses. The lym-

Fig 430. Section of rabbit kidney stained to show alkaline phosphatase as black material, apparently limited to the proximal convolutions. 100 ×. Courtesy of G. Gomori.

of branching ducts it seems best to consider the lobule as centering about these ducts rather than about the arterial tree. A similar problem is found in the determination of the hepatic lobule.

Interstitial Connective Tissue. The interstitial connective tissue in the normal kidney is very small in amount and is of the reticular type. Its branching fibers, accompanied by a few fibroblasts and fixed macrophages, form networks in the narrow spaces between the convoluted and straight tubules. The fibers are especially numerous and thick in the apex of the pyramids, where most of them are arranged concentrically around the ducts of Bellini and are embedded in an abundant, amorphous ground substance.

Thick, collagenous fibers are found only in the adventitia of the larger blood vessels. A few collagenous fibers surround the capsules of Bowman and the larger papillary ducts. Elastic fibers are absent except in the walls of the blood vessels. In old age the amount of collagenous tissue in the kidney may increase considerably through a transformation of the reticular fibers.

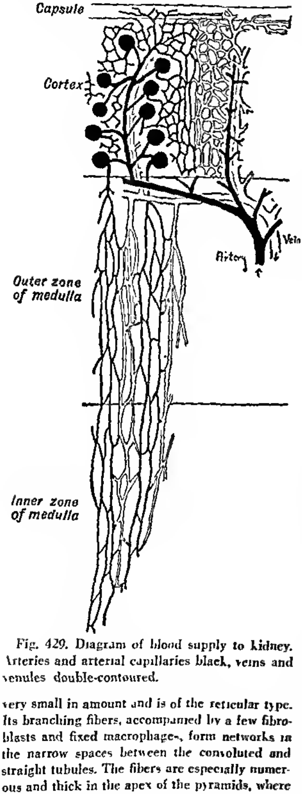

Fig. 429. Diagram of blood supply to kidney. Arteries and arterial capillaries black, veins and venules double-contoured.

Basement Membrane. The epithelium of the nephron and of the excretory ducts rests upon a well developed basement membrane which is hyalin in its central part. It is found in the renal corpuscle where it separates the endothelium of the capillary loops from the "visceral" epithelial layer (Fig. 422) and also lies beneath the parietal epithelium. In the proximal convolution the hyalin membrane is particularly thick. Its inner surface, to which the bases of the epithelial cells are attached, is provided with numerous, small, circular ridges. The interstitial reticular fibers fuse with the outer surface of the membrane.

Blood Vessels of the Kidney.

Practically all of the arterial blood of the kidney passes through the glomeruli before being distributed to the rest of the organ (see Figs. 421, 429).

The renal artery enters the hilus of the kidney and divides into two sets of principal branches, a ventral and a dorsal. The first set has a wider distribution in the organ than the second. These principal branches of the renal artery are "end arteries" and are not connected by large anastomoses. In the fat tissue surrounding the pelvis they branch into *interlobar arteries;* these are in the columns of Bertin between the Malpighian pyramids or lobes of the kidney.

The interlobar arteries break up into branches which run approximately parallel to the surface of the kidney at the limit between the bases of the pyramids and the cortex. They have a more or less arched course (arterial arcades) with the convexity directed toward the periphery of the organ. They are called *arciform* or *arcuate arteries.* At more or less regular intervals they give off smaller branches which run radially to the surface of the kidney. Since these radial branches are located in the cortical substance between

Phenol red has been observed to be eliminated by the proximal convolution in living frogs; the same part of the nephron in cultures from a human fetus of three and a half months secretes this indicator if it is added to the medium while the epithelium of the rest of the tubules does not.

As certain poisons more or less selectively affect particular portions of the proximal convolution, and as certain dyes and iron salts are eliminated in greater amounts in some parts of this segment of the nephron than in others, it is probable that different parts of the proximal convolution have different functions.

Richards and his co-workers have studied the functions of the various segments of the tubules by direct methods in the frog and salamander. Tubules were punctured at various levels and fluid removed for analysis, and fluid of known composition was perfused through a part of the tubule between two micropipettes. It was found that osmotically active constituents of the urine, chiefly sodium and chloride, are reabsorbed in the distal portions of the tubule, where acidification of the urine also takes place. Reabsorption of glucose takes place in the proximal portion in Necturus. Walker, Oliver and co-workers have begun similar studies on rat and guinea pig kidneys and found that reabsorption of reducing substances, creatinine and water from the glomerular filtrate takes place isosmotically in the proximal convolution. It also appears that the formation of hypertonic urine occurs in the distal part of the distal convolution through reabsorption of water.

Modern study of renal function has been greatly aided by the discovery that inulin, a complex carbohydrate, is filtered through the glomeruli in the same concentration as in the plasma, and is neither excreted by nor reabsorbed from the tubules. The *inulin clearance*, expressed as the number of cubic centimeters of plasma cleared of inulin per minute, gives a measure of *glomerular filtration*, and also a standard of comparison for other substances, by which it may be determined whether they are reabsorbed from or excreted by the tubules. Moreover, since *diodrast*, an organic iodine compound, when introduced into the blood in moderate concentrations, is completely removed from the blood chiefly by tubular excretion, during a single passage of the blood through the kidneys, the *diodrast clearance* gives a measure of the flow of blood through the kidney. When diodrast is present in the blood in concentrations too great to be removed from the plasma in a single passage through the kidneys, the clearance of this substance gives the maximal rate of tubular excretion and can be interpreted as a measure of tubular excretory mass.

Application of these methods for the study of renal function to normal and pathological kidneys is actively in progress. It is now established, for example, that creatinine, while neither excreted nor reabsorbed in the tubules in the dog and certain other species, is excreted into the tubules in man, and that urea is lost from the tubules, largely by passive diffusion into the capillaries.

A substance called *renin* has been extracted from the mammalian kidneys and found to have the property of raising the general arterial blood pressure. The relation of this material, and of substances which inactivate it, to hypertension is the subject of active study. Goldblatt summarizes the situation as of 1947 as follows: "*Renin*, an enzyme from the kidney, enters the blood stream through the renal vein, and acts upon *hypertensinogen*, a globulin in the blood plasma, to form *hypertensin*, a polypeptide, which is the active vasoconstrictor substance, and which can be inactivated by *hypertensinase*, an enzyme in the blood and in extracts of some organs." It is claimed that

phatics of the capsule join the lymph vessels of the neighboring organs. The lymph capillaries in the glandular tissue form dense networks between the uriniferous tubules, especially in the cortex. They pass into lymphatics which accompany the larger blood vessels and leave the kidney at the hilus. They are not present in the glomeruli or medullary rays.

Nerves. Macroscopic dissection shows that the sympathetic celiac plexus sends many nerve fibers into the kidney. Their distribution in this organ has not been worked out satisfactorily. It is relatively easy to follow nonmyelinated and myelinated fibers along the course of the larger blood vessels. They provide the adventitia with sensory nerve endings and the muscular coat with motor endings. Along with the afferent arterioles, nerve fibers may reach the renal corpuscles and some of them seem to form end branches on their surface. The nerve supply of the uriniferous tubules, however, has not been clearly demonstrated. Some investigators describe plexuses of fine nerve fibers which surround and seem to penetrate the basement membrane. On its inner surface they are supposed to form another plexus from which terminal branches arise to end with minute end knobs between the epithelial cells.

Histophysiologic Remarks. The kidneys in elaborating the urine eliminate water and some of the substances dissolved in the blood. With few exceptions they do not produce new material. In addition to the *excretory* functions of the kidneys, by which they dispose of waste products and substances foreign to the organism, they have equally important *conservative* functions by which they retain the necessary amounts of water, electrolytes, and other chemical substances in the body, while permitting any excess of these substances to be eliminated. By means of these latter functions they play a large part in the maintenance of the constancy of composition of the internal environment of the organism in the sense of Claude Bernard.

Two conclusions may be deduced safely from the structure of the organ: One is that the Malpighian corpuscle must act as a filtration apparatus with a high pressure in the capillaries. The other is that differences in structure of the several parts of the tubule must reflect differences in function.

Owing primarily to the brilliant experiments of Richards and his co-workers, it is now well established that the fluid aspirated from the glomerular space (with the rest of the nephron carefully blocked) in the living frog is identical in composition with the plasma except for the absence of fats, plasma proteins, and substances combined with these large molecules. This fluid contains water, phosphates, reducing substances, creatinine, uric acid, urea, and chloride; certain dyes are also found in this fluid after their introduction into the animal. That the glomerulus functions as an ultrafilter in mammals, including man, is supported by less direct but no less convincing evidence The passage of fluorescein and aesculin from the blood stream into the capsular space has been observed with the aid of ultraviolet light. Intravenously injected potassium ferrocyanide and uric acid can be demonstrated in the capsular space, but when injected into rabbits whose blood pressure had been lowered beneath the osmotic pressure of the blood neither of these substances is found within the nephron.

The observation that the circulation of blood may cease from time to time in particular glomeruli, although questioned recently, is probably correct.

It is also well established for a wide variety of animal forms that the renal tubules have both excretory and absorptive functions. That the tubules reabsorb certain substances, such as glucose, is shown by the fact that these substances are present in the glomerular filtrate in the same concentration as in the plasma, but are absent from the urine. There is now adequate evidence that tubular excretion plays an important part in the excretion of such substances as indigocarmin, phenol red, diodrast and creatinine in man.

sue. The upper part of the bladder is covered by the serous membrane of the peritoneum.

Although of mesodermal origin in the ureter and of entodermal in the bladder, the lining of the mucous membrane in all the parts just mentioned is the same "transitional" epithelium. In the calyces it is 2 to 3 cells thick, in the ureter 4 to 5, in the empty bladder 6 to 8 cell layers are seen. In the contracted condition of the wall of the viscus, the epithelium is thick and its cells are round, or even columnar or club-shaped. In the distended condition the epithelium is thin and the cells are greatly flattened and stretched parallel to the surface. Scattered lymphoid cells migrate between the epithelial cells. Owing to the similarity of the epithelial structure in these parts of the excretory tract, no conclusions can be drawn on the exact origin of epithelial cells found in the urine.

The epithelium lining the excretory passages seems to be impermeable to the normal, soluble substances of the urine. If the viscus is damaged this property may, of course, be greatly altered. *Intra-epithelial cysts*—round or oval cavities filled with a peculiar colloidal substance—often develop in the epithelium of the ureter and bladder. No true glands are present in the calyces, the pelvis, and the ureter; glands may be simulated here by small solid nests of epithelial cells located in the thickness of the epithelial sheet. In the urinary bladder, however, and in the vicinity of the internal urethral orifice, small, sometimes branched invaginations of the epithelium into the subjacent connective tissue can be found. They contain numerous clear mucus-secreting cells and are similar to the glands of Littré in the urethra.

No distinct basement membrane between the epithelium and the lamina propria can be discerned. The connective tissue of the latter, especially in the ureter, forms thin folds which may penetrate deep into the epithelium. The blood capillaries which they contain sometimes lie deep in the epithelial sheet.

The dense connective tissue of the mucous membrane generally does not form any papillae. It contains elastic networks and sometimes small lymphatic nodules. Its deeper layers have a looser arrangement and therefore the mucous membrane in the empty ureter is thrown into several longitudinal folds, which cause a festooned appearance of the edge of the lumen in cross sections (Fig. 431). In the bladder the deep looser layer of connective tissue is especially abundant, so that in the contracted condition of the organ the mucous membrane forms numerous, thick folds. In some places a thin layer of smooth muscle fibers seems to divide the connective tissue into a superficial *lamina propria* and a deeper submucous layer.

The muscular coat of the urinary passages generally consists of an inner longitudinal (Fig. 431, *ILM*) and an outer circular layer; beginning with the lower third of the ureter, a third external longitudinal layer is added, which is especially prominent in the bladder. In contrast to the intestine, the smooth muscles in the urinary passages do not form regular layers, but appear as loose, anastomosing strands, separated from one another by abundant connective tissue and elastic networks, which continue into the lamina propria mucosae.

In the small calyces, which are hollow cones capping the papillae of the pyramids, the strands of the inner longitudinal muscle layer end at the attachment of the calyx to the papilla. The outer circular strands reach higher up and form a muscular ring around the base of the papilla.

The calyces show periodic contractions moving from their base to their apex. This muscular activity helps to move the urine out of the papillary ducts into the calyces. The muscular coat of the ureter

the juxtaglomerular cells of the vas afferens undergo a rapid hyperplasia and elaboration of granules after partial clamping of the renal artery in rabbits and dogs, a procedure which results in an increased blood pressure. It has been suggested that these cells secrete a substance concerned with the maintenance of the increased arterial tension. But Selye and Stone believe the hypertensive materials are secreted by the tubular epithelium since the juxtaglomerular apparatus often disappeared completely in the kidney of rats after partial clamping of the renal artery.

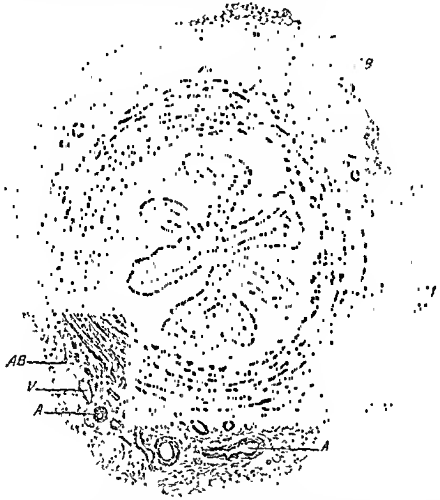

Fig. 431. Cross section of a markedly contracted ureter of a man: *A*, Arteries; *AB*, adventitial *connective tissue; ALM, external longitudinal muscle bundles; B, lamina propria; CM*, circular muscle bundles; *E*, deep layer of the epithelium, *F*, fat tissue; *ILM*, internal longitudinal muscle bundles; *OE*, superficial epithelial cells; *V*, veins. 30 ×. After Schaffer.

EXCRETORY PASSAGES OF THE KIDNEY

The excretory passages convey the urine from the parenchyma of the kidney to the outside. No appreciable changes in the composition of the urine occur in these passages except a slight admixture of mucus. In the uriniferous tubules no muscular elements are found. The walls of the excretory passages are provided with a well developed coat of smooth muscle; its contractions move the urine forward.

The calyces, the pelvis, the ureter, and the bladder all have a similar structure, although the thickness of the wall gradually increases in the sequence indicated. The inner surface is lined with a mucous membrane. There is no distinct submucosa in man and the lamina propria of the mucosa (Figs. 431, 433) is attached to the smooth muscle coat, which is covered by an adventitial layer of connective tis-

cells of which they form free end branchings provided with varicosities.

URETHRA

Male Urethra. The male urethra has a length of 18 to 20 cm. Three parts can be distinguished in it. The proximal, short part, surrounded by the prostate, is the *pars prostatica* (Fig. 438). Here the posterior wall of the urethra forms an elevation, the colliculus seminalis On its surface in the middle line the *vesicula prostatica* opens; to the right and to the left of this the two slitlike openings of the ejaculatory ducts and the numerous openings of the prostatic gland are located (p. 530). The second, also very short part (18 mm. long), the *pars membranacea urethrae*, stretches from the apex of the prostate to the bulb of the corpus cavernosum penis. The third stretch, the *pars cavernosa*, about 15 cm. long, passes lengthwise through the corpus cavernosum of the urethra.

The prostatic part is lined by the same "transitional" type of epithelium as the bladder. The pars membranacea and the pars cavernosa are lined by a stratified or pseudostratified columnar epithelium. Patches of stratified squamous epithelium are common in the cavernous parts; in the terminal enlarged part of the canal, the fossa navicularis, stratified squamous epithelium occurs as a rule. In the surface epithelium occasional mucous goblet cells may be found. Intraepithelial cysts containing a colloid-like substance are of common occurrence. Lymphocytes migrating through the epithelium are rare.

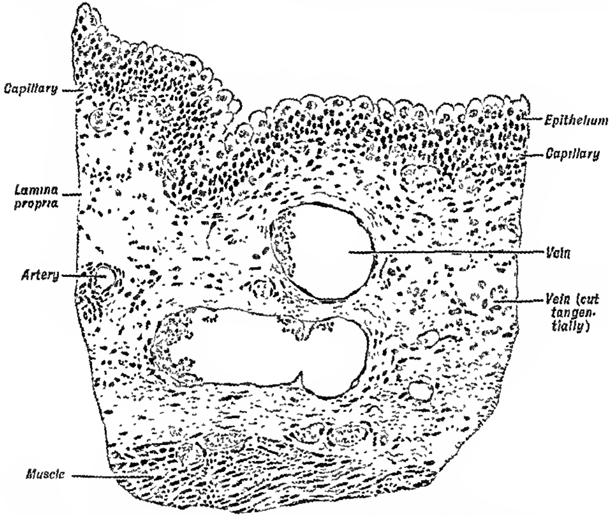

Fig. 433. Wall of human urinary bladder in contracted condition Capillaries penetrate the epithelium. 150 ×. (A.A.M.)

The lamina propria mucosae is a loose

also performs slow peristaltic movements. The waves of *contraction proceed from* the pelvis toward the bladder.

As the *ureters* pierce the wall of the *bladder obliquely, their openings are usually* closed by the pressure of the contents of the bladder and are open only when the urine is forced through them. A fold of the mucous membrane of the bladder acts as a valve and usually prevents the backflow of the urine. In the "intramural" part of the ureters, the circular muscular strands of their wall disappear and the connective tissue of the mucous membrane is occupied by longitudinal muscular strands whose contraction opens the lumen of the ureter.

The muscular coat of the *bladder* is very strong. Its thick strands of smooth muscle cells form three layers, which, however, cannot be distinctly separated from each other. The outer longitudinal layer is developed best on the dorsal and ventral surfaces of the viscus while in other places its strands may be wide apart. The middle, circular, or spiral layer is the thickest of all. The innermost layer consists in the body of the bladder of relatively rare, separate, longitudinal or oblique strands. In the region of the trigone, thin, dense bundles of smooth muscle *form a circular* mass around the internal opening of the urethra—the internal sphincter of the bladder.

Blood Vessels and Nerves. The blood vessels of the excretory passages penetrate first through the muscular coat and provide it with capillaries; then they form a plexus in the deeper layers of the mucous membrane. From here small arteries mount to the surface and form a rich capillary plexus immediately under the epithelium.

The deeper layers of the mucosa and the muscularis in the pelvis and the ureters contain a well developed network of lymph capillaries. In the bladder they are said to be present only in the muscularis.

In the adventitial and muscular coats of the ureter nerve plexuses, small ganglia and scattered nerve cells can be found. Most of the fibers supply the muscles; some fibers of apparently efferent nature have been traced into the mucosa and the epithelium.

A sympathetic nerve plexus, the plexus vesicalis, in the adventitial coat of the bladder is formed in part by the pelvic nerves which originate from the sacral nerves, and in part by the branches of the plexus hypogastricus. The vesical *plexus sends numerous nerves into the muscular coat.* A continuation of the nerve plexus, seemingly without nerve cells, however, is found in the connective tissue of the mucous membrane. Here the sensory nerve endings are located. Many fibers penetrate into the epithelium, between the

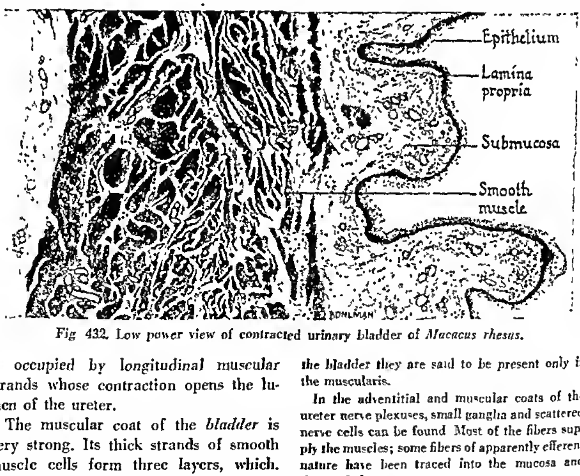

Fig 432. Low power view of contracted urinary bladder of *Macacus rhesus*.

The lamina propria, devoid of papillae, is a loose connective tissue with abundant elastic networks. It is provided with a highly developed system of venous plexuses and has, therefore, a cavernous character (corpus spongiosum).

The mucous membrane with its veins is surrounded by a thick mass of smooth muscles; the inner layers of the latter have a longitudinal, the outer layers a circular arrangement. Distally the smooth muscles are strengthened by a striated muscle sphincter.

Histogenesis of the Kidney. The independent origin of the nephrons and the collecting tubules in the embryo has been mentioned. The nephrons develop through differentiation of a compact, *mesenchyme-like tissue, the metanephric blastema,* which arises from the mesoderm. The system of excretory ducts arises as a hollow outgrowth of the wolffian duct, in much the same manner as other epithelial glands develop. This outgrowth grows forward in the mesenchyme as *the primordium of the ureter and of the renal pelvis.* It forms four branches—the primordia of the calyces, which end blindly in club-shaped dilatations, each of which forms secondary, tertiary, etc., branches—the collecting tubules of various orders.

In human embryos of 7 mm. the metanephric blastema adheres to the wall of the ...ted pelvis

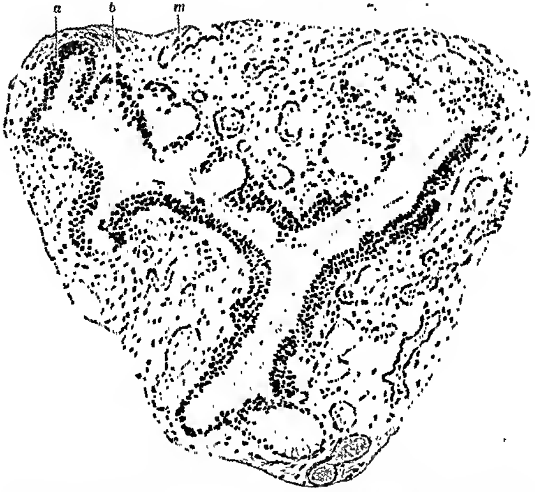

Fig 435 Urethral gland (gland of Littré) from the pars cavernosa urethrae of man: *a,* Darkly staining, stratified columnar epithelium, *b,* epithelium with clear cells; *m,* outpocketings with clear mucous cells; *bv,* blood vessels. 187 ×, reduced to 9/10. (A.A.M.)

primordium and appears in sections as a semilunar *cap.* As the branches of the pelvis form, the metanephric cap separates into a piece for each branch (human embryos of 9.5 mm.), so that the ampullar dilatation of each branch carries its own metanephric cap.

In human embryos 13 to 19 mm. in length, the edges of the cap covering each blind end of the collecting tubules swell and glide down its sides. *As the ampulla divides dichotomously the metanephrogenic cap is divided equally between the two new ampullae.*

connective tissue with abundant elastic networks; no separate submucous layer can be distinguished. This connective tissue contains numerous scattered bundles of smooth muscle, mainly longitudinally directed. In the outer layers, however, circular bundles are also present. The lamina propria has no distinct papillae; the latter appear only in the fossa navicularis. The membranous portion of the urethra is surrounded by a mass of striated muscle, a part of the urogenital diaphragm. surface of the mucous membrane, but in many places this epithelium is transformed into compact intra-epithelial nests of clear cells, which give the reaction of mucus. In old age some of the recesses of the urethral mucosa may contain concretions similar to those of the prostate.

The deeper venous plexuses of the urethral mucosa in the pars cavernosa gradually merge into the cavernous spaces of the spongy body (p. 537). Numerous sensory nerve endings are present in the urethral mucous membrane.

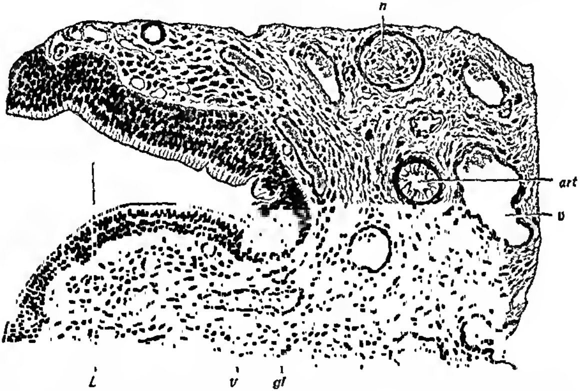

Fig. 434. Section of the cavernous part of the male human urethra: *L*, Lacuna; *gl*, intra-epithelial group of glandular cells, *v*, veins; *art*, artery; *n*, encapsulated sensory nerve ending. 187 ×, reduced to 9/10. (A.A.M.)

The surface of the mucous membrane of the urethra shows many recesses, the *lacunae of Morgagni*. These outpocketings continue into deeper, branching tubules, the *glands of Littré* (Fig. 435); the larger ones among them are found especially on the dorsal surface of the cavernous portion of the urethra. They run obliquely in the lamina propria and are directed with their blind end toward the root of the penis; they sometimes penetrate far into the spongy body. The glands of Littré are lined with the same epithelium as the

Female Urethra. The female urethra is 25 to 30 mm. long. The mucous membrane forms longitudinal folds and is lined with stratified squamous epithelium; in many cases, however, pseudostratified columnar epithelium can be found. Numerous invaginations are formed by the epithelium; the outpocketings in their wall are lined in many places with clear mucous cells, as in the glands of Littré of the male urethra. The glands may accumulate colloid material in their cavities or may even contain concretions.

Cameron and Chambers (1938) and Flexner (1938).

The terminal branches of the collecting tubules and the nephrons may sometimes miss each other and remain disconnected. In such cases the convoluted tubules with their renal corpuscles continue for some time to elaborate urine and, having no outlet, are gradually transformed into cysts. The cystic kidney is a not uncommon abnormality of development.

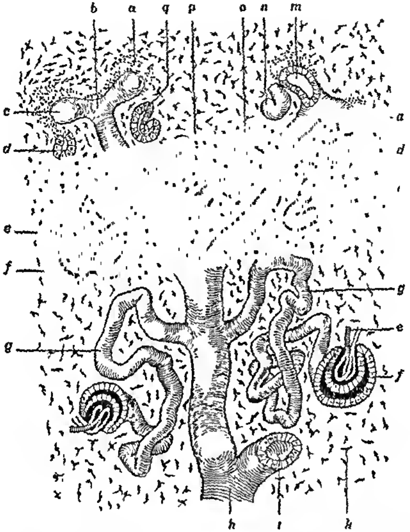

Fig 437. Diagram of the development of the metanephros: *a*, Metanephrogenic tissue capping the ampulla of the collecting tubule; *b*, T-shaped branching of the growing end of a collecting tubule; *c*, enlarged blind end of same (ampulla); *d*, primordium of uriniferous tubule just formed from the metanephrogenic tissue; *e*, vessel which forms the glomerulus, *f*, capsule of Bowman cut open; *g*, uriniferous tubule in later stage of development; *h*, collecting tubule; *i*, cross section of same, *k*, connective tissue; *l*, uriniferous tubule before the establishment of a connection with the collecting tubule; *m*, ampulla of collecting tubule cut open; *n*, primordium of uriniferous tubule just separated from metanephrogenic tissue; *o*, newly formed uriniferous tubule which has just become connected with collecting tubule (stage immediately following *l*); *p*, spoon shaped enlargement of the blind end of a uriniferous tubule—capsule of Bowman; *q*, primordium of uriniferous tubule just separated from metanephrogenic tissue (same as *n*), cut open. Modified after Corning.

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The roundish, compact metanephric body soon acquires an eccentric lumen around which its cells become radially arranged; it is now called the metanephric vesicle. Next it stretches and is transformed into an S-shaped tubule which grows rapidly in length and becomes tortuous. It is the future nephron. One end of it coalesces with and opens into the neighboring collecting tubule. The other end enlarges, flattens slightly and is invaginated by a tuft of capillaries from a branch of the renal artery. In this way the Malpighian or renal corpuscle is formed with its is much thicker than the parietal. Later both layers become simple squamous epithelia. Soon after the S-shaped tubule of the nephron becomes connected with the collecting tubule, the histologic differentiation of the different parts of the nephron begins. The proximal part, adjacent to the capsule, becomes tortuous and its epithelium develops a glandular character, increases in height, and its cytoplasm stains with acid dyes. The following stretch of the tubule forms a loop, which slips out of the coils formed by the convoluted tubule and extends toward the renal

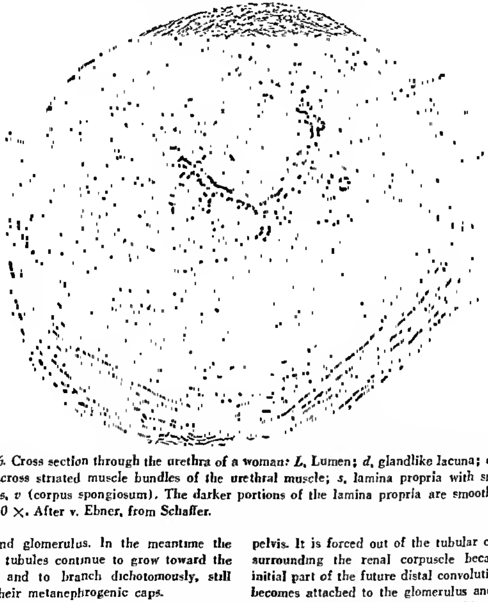

Fig. 436. Cross section through the urethra of a woman: *L*, Lumen; *d*, glandlike lacuna; *e*, epithelium; *m*, cross striated muscle bundles of the urethral muscle; *s*, lamina propria with small and large veins, *v* (corpus spongiosum). The darker portions of the lamina propria are smooth muscle bundles. 10 ×. After v. Ebner, from Schaffer.

capsule and glomerulus. In the meantime the collecting tubules continue to grow toward the periphery and to branch dichotomously, still keeping their metanephrogenic caps.

Succeeding generations of nephrons with their renal corpuscles are added to the branching tree of the collecting tubules, until the whole metanephric blastema is exhausted. This continues in the human fetus throughout the latter period of intra-uterine life and comes to its end six or eight days after birth.

When the glomerulus invaginates the wall of the capsule of Bowman the visceral epithelium pelvis. It is forced out of the tubular convolute surrounding the renal corpuscle because the initial part of the future distal convolution early becomes attached to the glomerulus and consequently cannot be removed from this place as the nephron grows in length. The epithelium of the collecting tubules soon acquires its typical clear appearance; this differentiation begins in the deeper parts, which are nearer to the pelvis, and gradually extends peripherally. The evidences of beginning function in the developing embryonic mammalian kidney have been studied experimentally by Gersh and Flexner (1937).

Chapter XXIV

MALE GENITAL SYSTEM

The male reproductive system consists of the testes, a complex system of excretory ducts with their auxiliary glands, and the penis.

THE TESTIS

The testis is a compound tubular gland, surrounded by a firm, thick white capsule, the albuginea testis—a typical fibrous membrane (Fig. 439). At the posterior edge of the organ, the thickening of the capsule projects into the gland as the *mediastinum testis*. From the mediastinum thin partitions, the *septula testis*, extend radially to the capsule and divide the organ into about 250 conical compartments, the *lobuli testis*, which converge with their apices toward the mediastinum. As the septula are interrupted in many places, the lobules intercommunicate, especially in their peripheral portions.

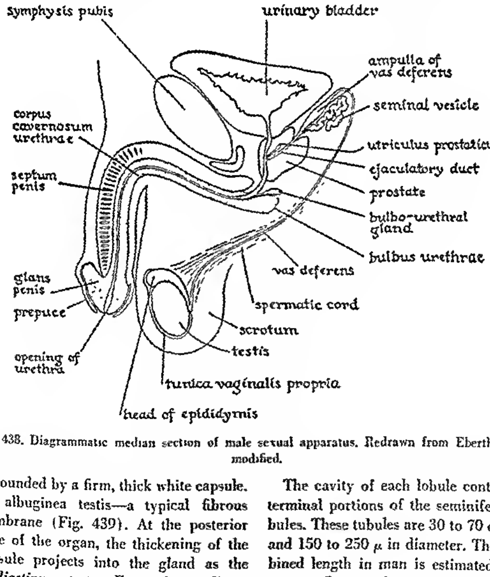

Fig. 438. Diagrammatic median section of male sexual apparatus. Redrawn from Eberth, slightly modified.

The cavity of each lobule contains the terminal portions of the seminiferous tubules. These tubules are 30 to 70 cm. long and 150 to 250 μ in diameter. Their combined length in man is estimated at 250 meters. One to three of them occupy a lobule. They have an extremely tortuous

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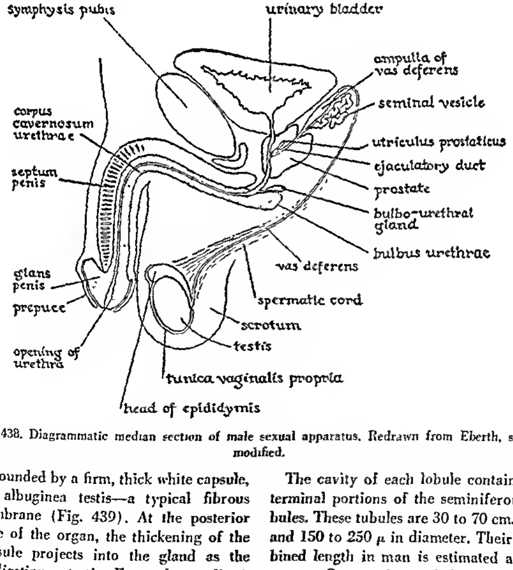

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course, rarely branch, and are called the *convoluted seminiferous tubules* (Fig. 440). The tubules in adjacent lobules may be connected by loops. The spermia are formed in the convoluted tubules. The testes are suspended in the scrotum by the spermatic cords. Each of these contains the excretory duct (*ductus deferens*), blood vessels and nerves supplying the testis on that side of the body. The epi-

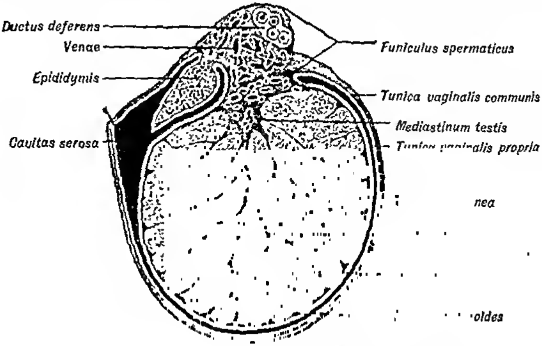

Fig. 439. Cross section of human testis with its envelopes. 2 ×. After Eberth.

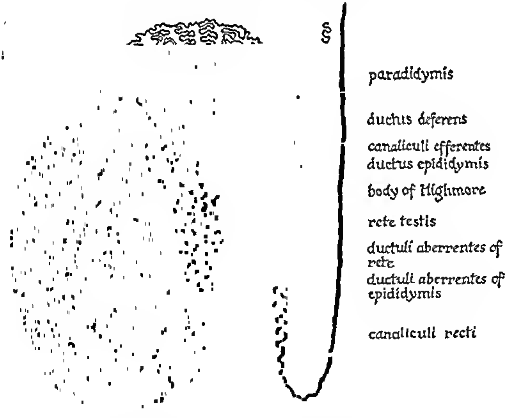

Fig 440. Diagram of the arrangement of the seminiferous tubules and the excretory ducts in the testis and epididymis x, Communication between seminiferous tubules of different lobules

didymis, an elongated body attached to the posterior surface of the testis, contains the proximal parts of the excretory duct system of this organ.

The anterior and lateral surfaces of each testis and epididymis are surrounded by a cleftlike serous cavity, a detached part of the peritoneal cavity, in the dorsal wall of which the testes develop in the embryo before descending into the scrotum. The wall of this cavity, the *tunica vaginalis propria testis*, comprises an outer parietal, and an inner visceral layer. At the posterior edge of the testis, where the epididymis is attached and the blood vessels and nerves enter the organ, the parietal layer is reflected into the visceral layer. After removal of the parietal layer, the *visceral coat* covering the testis appears as a free, smooth surface. It is lined with mesothelium. This is the remnant of the germinal epithelium which covers the surface of the primordium of the gonad in the embryo and gives rise to the glandular tissue of the testis. The *tunica vaginalis propria* enables the testis, which is very sensitive to pressure, to glide freely in its envelopes. Obliteration of the serous cavity of the tunica vaginalis causes atrophy of the testis.

SEMINIFEROUS EPITHELIUM

In the adult the convoluted seminiferous tubule is lined by the complex seminiferous epithelium with its two kinds of cells. The first are nutrient and supporting elements—the *sustentacular cells* (*of Sertoli*). The others, forming the vast majority, are the *sex* (*germ* or *spermatogenic*) cells, which, through proliferation and complex transformations, furnish the mature spermia.

Cells of Sertoli. In a tubule with active spermatogenesis the Sertoli cells are slender, pillar-like elements perpendicular to the basement membrane to which they are attached. They are separated from one another at fairly regular intervals by the densely crowded spermatogenic cells (Fig. 442).

The outlines of the sustentacular cells cannot be seen distinctly and there is a widespread opinion that the spermatogenic cells are embedded in a continuous "Sertolian syncytium." However, in sections parallel to the basement membrane, the bases of the Sertoli cells are sometimes seen as distinctly outlined, polygonal areas. In pathologic conditions, when the spermatogenic cells degenerate and disappear to a large extent, the lumen of the depleted seminiferous tubules appears surrounded by a loose protoplasmic network with scattered Sertoli nuclei (Fig. 441), and the few spermatogenic cells which escaped destruction. Occasionally a Sertoli cell may round off and float freely in the enlarged lumen where it may phagocytose degenerating spermatogenic cells or spermia.

The characteristic nucleus of the Sertoli cell has an oval shape and an average size of 9 by 12 μ. In the human testis it is usually at some distance from the basement membrane with its long axis directed radially. The membrane of the Sertoli nucleus is usually wrinkled and the folds often extend deep into the interior of the nucleus (Fig. 443). This is probably not a sign of amitotic division. The vesicular nucleus is in striking contrast to the nuclei of the spermatogenic cells. The nucleus contains a large, compound nucleolus which consists of an oval, central, acidophil part and of 1 to 3 small, round, basophil granules.

The cytoplasm of the Sertoli cells in fixed preparations has a loose reticular structure. It contains small mitochondria, wavy fibrils, granules staining with iron hematoxylin, and lipoid droplets which cause the brown color of the sectioned surface of the fresh testis and are supposed to be an evidence of the nutrient activity of these cells. In the human testis each Sertoli cell contains one spindle-shaped crystalloid near the nucleus.

At certain periods during spermatogenesis, the Sertoli cells enter temporarily into an intimate connection with the developing spermatogenic cells adjacent to the basement membrane.

Under normal conditions the Sertoli

cells are never seen to divide either mitotically or amitotically. They are highly resistant toward various noxious factors which easily destroy the spermatogenic cells.

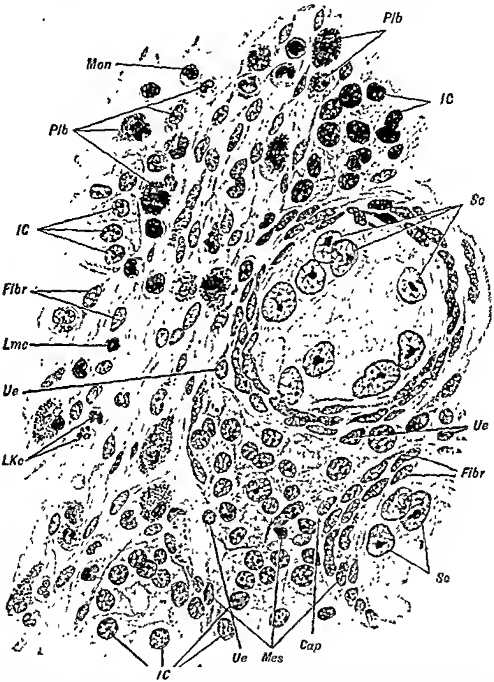

Fig. 441. Portion of a seven months' cryptorchid testis of an adult guinea pig which had been stained vitally with lithium carmine several days before death. The spermatogenic cells have disappeared and the tubules are filled with a syncytium of Sertoli cells (*Sc*); the interstitial cells (*IC*) have increased in number and do not store carmine; *Cap*, capillary; *Fibr*, fibroblasts; *LKc*, leukocytes; *Lmc*, lymphocyte; *Mes*, undifferentiated mesenchymal cells; *Mon*, monocyte; *Plb*, polyblasts storing carmine; *Ue*, transition from mesenchymal cells to interstitial cells. Hematoxylin stain. 490 ×. After Esaki.

Spermatogenesis. General Remarks. There are two phases in the process of spermatogenesis. In the first phase, *spermatocytogenesis*, the germ cells undergo repeated mitoses and certain structural changes, and give rise to a new type of cell (spermatid) which contains only one half the somatic chromatin mass in its (haploid) nucleus and is unable to divide

In the second phase, *spermiogenesis,* the spermatids undergo a series of complex transformations which result in the formation of the mature *spermia* or *spermatozoa* (see Fig. 450). They do not resemble other cells of the organism and have specific forms for different species.

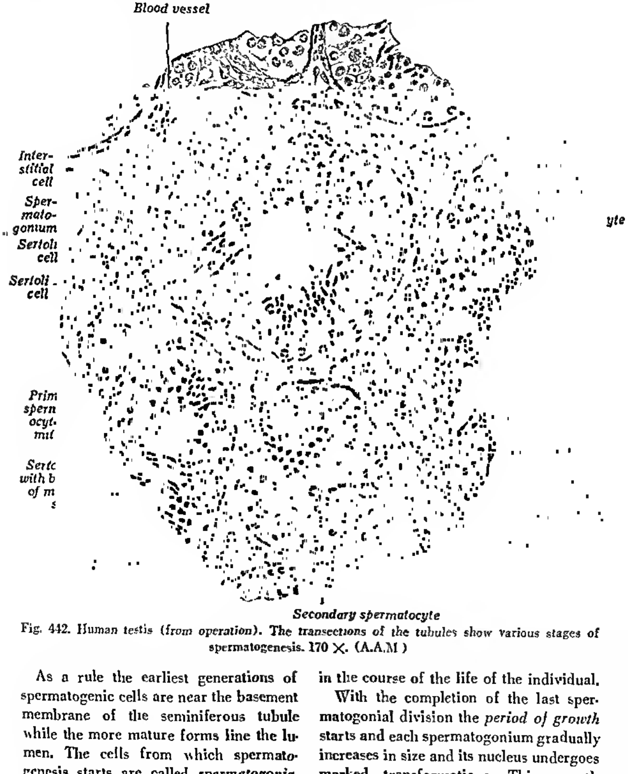

Fig. 442. Human testis (*from operation*). The transections of the tubules show various stages of spermatogenesis. 170 ×. (A.A.M.)

As a rule the earliest generations of spermatogenic cells are near the basement membrane of the seminiferous tubule while the more mature forms line the lumen. The cells from which spermatogenesis starts are called *spermatogonia.* They divide mitotically, but the number of divisions is not known. This first step in spermatocytogenesis is the *period of proliferation.* During this stage some of the spermatogonia remain unchanged and keep their position along the inner surface of the basement membrane. They are the source of the countless spermia produced in the course of the life of the individual.

With the completion of the last spermatogonial division the *period of growth* starts and each spermatogonium gradually increases in size and its nucleus undergoes marked transformations. This growth causes a further shifting of the cells to-

ward the lumen of the tubule. The growing cell is known as a *primary spermatocyte*. When it has reached its full development the *period of maturation* begins and the primary spermatocyte divides into two new cells—the *secondary spermatocytes*. Each secondary spermatocyte soon divides, giving rise to two *spermatids*. They are the last generation of spermatogenic cells. By individual transformations they become spermia.

The two mitoses occurring in rapid succession and leading from one primary spermatocyte to four spermatids are of a peculiar character and differ from the common somatic mitoses. They are called "mitoses of maturation," or "meiotic divisions" and through them the nucleus of each spermatid receives only one half of the somatic number of chromosomes typical for the respective animal species (48 in man).

Spermatocytogenesis. The nucleus in the earlier generations of spermatogonia contains dustlike particles of chromatin and a round body that stains like chromatin. These early spermatogonia are called "primary spermatogenic cells" or "spermatogonia with dustlike nuclei." The cytoplasm contains granular mitochondria. Near the nucleus are a pair of centrioles and a thin *crystalloid body* smaller than that of the Sertoli cells.

The later generations of spermatogonia are smaller and are found either at the basement membrane or a short distance from it toward the lumen. The crystalloid seems to be absent. In the nucleus the chromatin is arranged in darkly staining flakes on the inner surface of the membrane—hence the name of "spermatogonia with crustlike nuclei." These differences between the earlier and the later generations of spermatogonia are not very distinct in man.

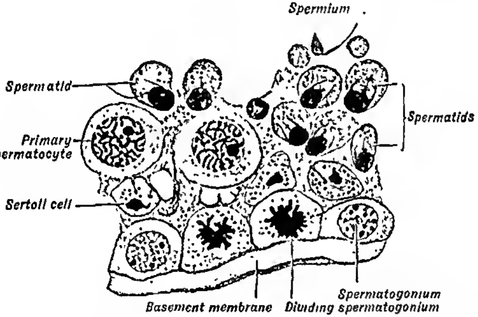

Fig. 443. Human testis, from young adult. Seminiferous epithelium with mitoses of spermatogonia. The spermatids show a caudal sheath. Iron-hematoxylin stain. 740 ×. (A.A.M.)

The mitoses of the spermatogonia show the somatic number of chromosomes characteristic of the species. This number is forty-eight in man. In the dividing spermatogonium, the chromosomes consist of twenty-three pairs of varying sizes and shapes and of one pair of heterochromosomes (X-Y). During the spermatogonial mitoses, all of the chromosomes, including the heterochromosomes, split longitudinally in the usual way. The X and Y chromosomes in man were first accurately figured by Painter (1923) although the

claim of an X·Y condition in the male had previously been made by others.

The changes undergone by a spermatogonium developing into a primary spermatocyte (period of growth) represent a gradual preparation for the meiotic divisions and the reduction of chromatin.

The primary spermatocytes move toward the lumen of the tubule and occupy the middle zone of the seminiferous epithelium. As their transformations extend over a considerable period of time, these cells are numerous in the seminiferous epithelium and show considerable variations in size and structure.

The fully developed primary spermatocytes are large, spherical or oval cells. The long axis of the oval cells, when they are closely packed together (rat), is perpendicular to the basement membrane. The nucleus is also large; its structure undergoes a series of typical gradual transformations which begin at once after the completion of the last spermatogonial mitosis and finally lead to the first meiotic division.

In the first meiotic division of the human spermatocytes the X·Y chromosome separates, the X going to one daughter cell and the Y to the other. Thus, of the two secondary spermatocytes originating from a dividing primary spermatocyte, one will contain twenty-three ordinary chromosomes and an X chromosome, while the other will have twenty-three chromosomes and the Y. Thus, there are two kinds of secondary spermatocytes.

The daughter cells of the secondary spermatocytes, the *spermatids*, are relatively small, spherical cells. Each contains a spherical nucleus about 5 to 6 μ in diameter, with several darkly staining chromatin granules. Half of the total number of young spermatids contain the X elements and half the Y.

The following pages contain further details of these complex cytological changes. The chromatin flakes or crusts on the membrane of the spermatogonial nucleus break up into small granules which assemble to form long, thin, tortuous chromatin threads—the *leptotene stage*. During this stage the chromosomes, while still arranged in the form of long thin threads, conjugate two by two, probably side by side, and form a haploid number of bivalent chromosomes (*parasynapsis*). Later, the thin filaments of chromatin become shorter and thicker (*pachytene stage*) and acquire a granular appearance; finally they may show a more or less distinct longitudinal split, as an indication of their origin through conjugation (*diplotene stage*). The filaments then contract into bivalent chromosomes, each consisting of two synaptic mates. Each of these, sooner or later, may undergo a longitudinal split—a preparation for the second meiotic divi-

Fig. 444. Human spermatogonial plate showing 48 chromosomes. Iron-hematoxylin stain. 3600 ×. After Evans and Swezy.

sion. Thus, the bivalent chromosomes consist of four parts and are called tetrads. In mammals the history of their development is not as clear as in many invertebrates. The mammalian tetrads are usually plump, irregular, polyhedral bodies, and show, as a rule, only one split.

Besides the mitochondria the cytoplasm has a cytocentrum consisting of a pair of centrioles in the large attraction sphere, which in sex cells is usually called the *idiozome*. It is possible, however, that the idiozome is a structure specific for the sex cells. The periphery of the idiozome is surrounded by peculiar rodlets, sometimes forming a net—the Golgi apparatus.

When the first meiotic mitosis begins, the centrioles move away from each other and between them the achromatic spindle is formed. The nuclear membrane and the nucleolus disappear, and the bivalent chromosomes or tetrads are arranged on the equator of the spindle. Simultaneously the idiozome and the Golgi body

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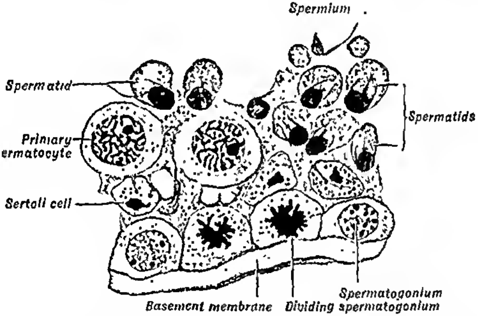

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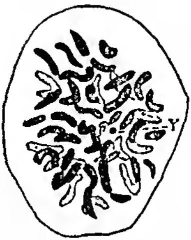

Fig. 414. Human spermatogonial plate showing 48 chromosomes. Iron-hematoxylin stain. 3600 ×. After Evans and Swezy.

Thus, the bivalent chromosomes consist of four parts and are called tetrads. In mammals the history of their development is not as clear as in many invertebrates. The mammalian tetrads are usually plump, irregular, polyhedral bodies, and show, as a rule, only one split.

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break up into fragments (called *dictyosomes*) which collect around the poles of the spindle. During the metaphase, when the equatorial plate is seen from the pole, the chromosomes can be counted. In the human spermatocyte monaster the primary spermatocyte divides into two dyasters, each bivalent chromosome or tetrad separates into its two constituent parts, along the line of their previous conjugation. These parts correspond to the common, single chromosomes

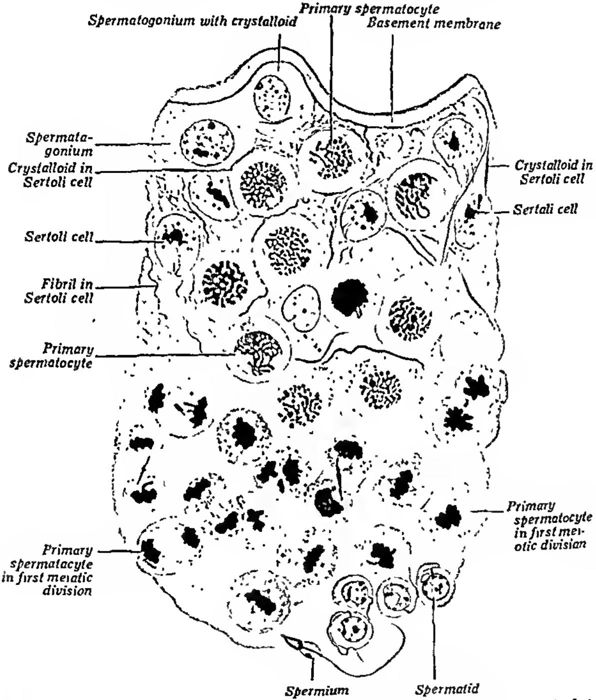

Fig. 445. Same testis as in Fig. 412 Seminiferous epithelium with primary spermatocytes in first meiotic division. Iron-hematoxylin stain. 1000 ×, reduced to ¾. (A.A.M.)

twenty-four bivalent chromosomes can be distinguished. Ten have the form of irregular rings; thirteen are small and sometimes show an indistinct split. The twenty-fourth chromosome, of an elongated, club-shaped form, is likewise bivalent; it is the X-Y *chromosome*.

During the anaphase, when the monaster of While they recede to the poles of the spindle they may show (in some of the lower vertebrates) a distinct longitudinal split—an anticipation of the impending second meiotic division. They are called the *dyads*.

The difference between mitosis and meiosis is essentially this: In a somatic mitosis the indi-

vidual chromosome splits and the half chromosomes separate, while in meiosis whole chromosomes, the *synaptic mates* (the halves of the bivalent chromosomes) move away from each other toward the poles. This peculiar process has been named *heterotypic mitosis.*

While in many lower animals the second meiotic division starts immediately after the first, without giving the nuclei time to return to the resting condition, in mammals there is a distinct *interkinetic (resting) stage.* Its duration is unknown. The body and the nucleus of the secondary spermatocytes are smaller than in the primary spermatocytes. When the reconstruction after the first division is completed, the nucleus contains an indistinct network with round or angular chromatin granules, but no distinct nucleolus. The constituents of the protoplasm seem to be the same as in the primary spermatocyte

In the second meiotic division, the spindle with the centrioles on the poles is less prominent and more slender than in the first mitosis and extends with its apices nearly to the surface of the cell. The idiozome and Golgi body seem to divide in the same way as in the first division. The chromosomes (twenty-four), which are now small and dumbbell-shaped, are arranged on the equator of the spindle and each divides into halves which are believed to be the products of a longitudinal split of the respective chromosome. Thus, the second meiotic division corresponds in principle to an ordinary somatic mitosis, and is, therefore, sometimes called the "homeotypic division."

The cytoplasm contains the usual granular mitochondria, one or several darkly staining "chromatoid bodies," the idiozome with the Golgi apparatus and the centrioles. The idiozome has now separated from the centrioles and is a darkly staining, spherical body with a central clear area—the so-called *acroblast.* The centriole, first located near the nucleus, later divides and the new pair of centrioles thus formed at once recedes to the periphery of the cell. Here the two granules arrange themselves in such a way that the line connecting them is perpendicular to the surface of the cell The distal centriole touches the cell surface, while the proximal one is located deeper in the cytoplasm.

The different structural details of spermatocytogenesis just described, as well as the phenomena of spermiogenesis, discussed below, cannot be considered as completely elucidated for human material For obtaining a general idea of the process, a comparative study of the testis of various animal species, including invertebrates, is necessary. (See also Gatenby and Beams.)

Spermiogenesis. The spermatids are the last generation of spermatogenic cells. They do not divide and each one has to

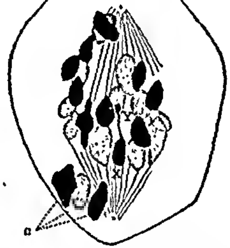

Fig. 416 Spindle of a human primary spermatocyte showing twenty-four chromosomes. The chromosomes at *a* were behind the others and were drawn outside the spindle for clearness. Iron-hematoxylin stain. 3600 ×. After Evans and Swezy.

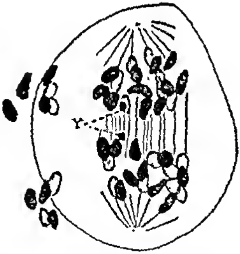

Fig. 417. Anaphase of a human secondary spermatocyte showing the two small Y chromosomes—the last to divide. There are twenty-four chromosomes in each group; those at the left were behind the others and were drawn separately for clearness. Iron-hematoxylin stain. 3600 ×. After Evans and Swezy.

undergo a long series of peculiar transformations, deeply affecting every constituent of the cell, before the mature sper-

mium or spermatozoon is formed (Fig. 450).

The most striking changes occurring at the beginning of spermiogenesis concern the *centrioles and the acroblast*. From the distal centriole, which is located at the surface of the cell body, a thin *filament grows out* (Fig. 450, *b*). It represents the primordium of the axial thread of the tail or flagellum of the spermium. At first it is coiled over the surface of the cell. Later it grows longer and thicker and straightens out.

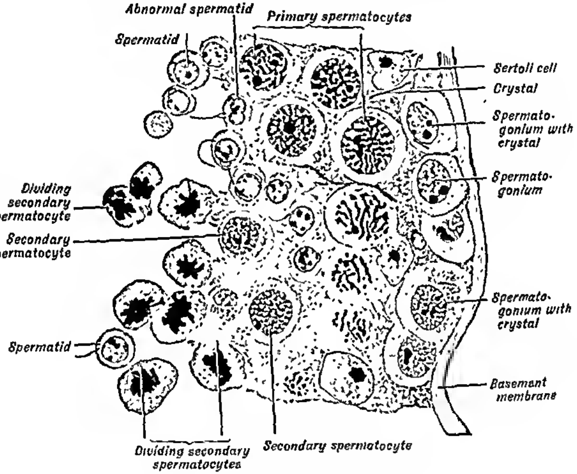

Fig. 448. Same testis as in Fig. 442. Seminiferous epithelium with mitoses of secondary spermatocytes—second meiotic division 1000 ×, reduced to ¾. (A.A.M.)

The acroblast becomes attached to the surface of the nucleus, marking its anterior pole. In the interior of the clear central area of the acroblast a small darkly staining, round body—the *acrosome*—appears. Then the acroblast, flattening against the surface of the nucleus, assumes a hemispherical shape and the acrosome comes in direct contact with the nuclear membrane. The latter in this place may also appear flattened or even *invaginated. The darker peripheral layer of* the acroblast, which consists of Golgi material, recedes into the protoplasm as the so-called *acroblast remnant* and later disintegrates. The clear layer, covering the acrosome, is reduced in thickness and extends with its edge backward, passing beyond the equator of the nucleus. In the human spermium it forms a very delicate, thin membrane, the *head cap*, which covers the anterior pole of the nucleus, the head of the future spermium. The posterior edge of the cap can be seen as a thin line, running transversely across the head.

The nucleus, which at first is flattened or even invaginated at its anterior pole by the acroblast with the acrosome, later regains its convexity. It moves toward the periphery of the cell body, until it touches (Fig. 450, *d*) and later, even evaginates the cell surface by its capped anterior pole (e, *f*). Then it begins to shrink and assumes a slightly flattened, oval form (*g*), while the chromatin gradually condenses into a darkly staining homogeneous mass. The anterior half of the nucleus stains somewhat lighter than the posterior. In this way the *head of the spermium* is formed.

During the described transformations of the nucleus, at the time when it begins to move to the anterior pole of the cell, a peculiar temporary structure appears in connection with the nucleus —the *caudal sheath* (Fig. 450, e, *f*). It is a thin

membrane which has the form of a wide tube or funnel. It begins at the equator of the nucleus and extends a short distance backward, where it ends with a free edge in the cytoplasm. It surrounds the posterior pole of the nucleus, the black. Keeping their position in relation to each other, they move from the periphery of the cell body toward the surface of the nucleus. When the proximal centriole becomes definitely attached to the latter, it marks the posterior pole

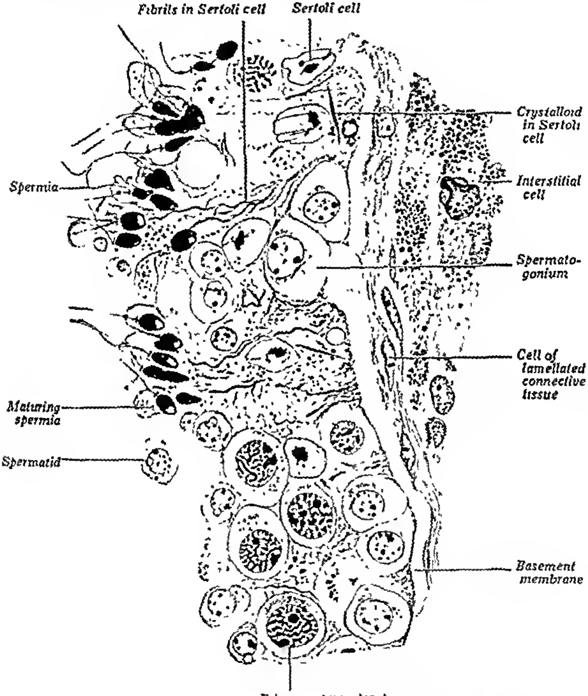

Fig. 449. Same testis as in Fig. 442 Seminiferous epithelium with bunches of maturing spermia, connected with Sertoli cells Iron-hematoxylin stain. 1000 ×, reduced to ¾. (A.A.M.)

centrioles and the anterior part of the axial filament. Its significance is obscure and it soon disappears (Fig. 450, g, h).

The centrioles undergo important transformations. These are best followed in iron-hematoxylin sections, where the centrioles are stained of the nucleus or of the future head, while the anterior pole, as has been explained, is marked by the acrosome. Thus the nucleus acquires a distinctly polar differentiation.

The proximal centriole, attached to the nuclear membrane, grows out asymmetrically in the

transverse direction and, according to its position, is seen as a granule or a rodlet. It is transformed into the sometimes double, anterior knob of the neck of the spermium. The distal centriole is usually seen as a short, transverse rod behind the anterior (proximal) centriole. Then the distal centriole divides into two parts. Its anterior part remains in its place, separated from the proximal centriole by a very small amount of lightly colored substance and constitutes the posterior knob of the neck. The axial thread remains connected with this body. The posterior part of the distal centriole acquires the shape

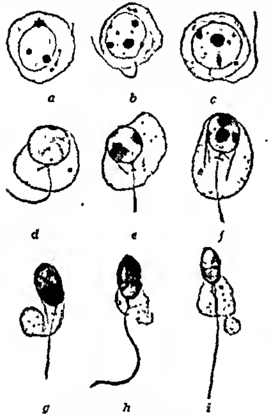

Fig. 450. Nine successive stages of spermiogenesis from human testis, explanation in the text, page 514. Iron-Hematoxylin stain. 1500 ×. (A.A.M.)

of a ring surrounding the axial thread (Fig. 450, *h*). It moves away from the posterior knob, gliding along the axial filament, until it reaches the surface of the cell body. Its position marks the posterior limit of the *middle piece* of the spermium (*i*).

During the migration of the centrioles toward the nucleus the axial filament is partly drawn into the interior of the protoplasm. Thus, an intra- and an extracellular part of the thread can be distinguished. The second grows in length and finally reaches the size of the tail of the spermium. It seems to secrete on its surface an extremely thin sheath which is missing on the end section of the tail.

The mitochondria of the spermatid assemble around that part of the axial thread extending between the posterior knob and the ring. They seem to fuse together into a heavily staining filament, surrounding the axial thread in a spiral coil—the *spiral sheath* of the middle piece of the spermium.

Concurrently with the transformations just described, the cytoplasm of the spermatid gradually recedes from the nucleus, which protrudes more and more at the anterior pole of the cell and acquires an elongated, pearlike shape. It contains, besides some mitochondria not included in the spiral sheath and the above-mentioned disintegrating acroblast remnant, fat and lipoid droplets and heavily staining granules of undefinable nature (remnants of the chromatoid body, etc.). For a certain time the cytoplasm remains attached to the middle piece of the young spermium; it probably furnishes an external sheath for this part. Finally, it is sloughed off as an irregular round mass and disintegrates in the lumen of the seminiferous tubule. A part of this granular detritus is carried into the semen. The major part, however, seems to be absorbed and utilized by the Sertoli cells. A small drop of cytoplasm remains attached to the middle piece of the spermium for a long time.

Mature Spermium. The mature human spermium consists of a head, a connecting or middle piece, and a tail. In ordinary sections the spermia do not show any particular inner structure. For seeing the details, special histologic methods such as iron hematoxylin and highest magnifications are necessary.

The *head* is a flattened, almond-shaped body measuring 4 to 5 μ in length and 2.5 to 3.5 μ in width. It is a condensed nucleus. The middle piece is of cylindrical or spindle shape and connects the posterior pole of the head with the tail. It has a length of 5 μ and a thickness of 1 μ. The tail has a length of 52 μ. At its anterior end it has the same thickness as the middle piece but gradually tapers down toward the free end. It can be subdivided into the principal part and a short terminal part of extreme thinness.

In fresh preparations from the testis the spermia are either motionless or display only a slight motility.

In man, two kinds of spermia are produced in equal numbers—spermia containing in their head the chromatin of 23 chromosomes and an X chromosome, and spermia with 23 chromosomes and a Y chromosome. All mature egg cells, however, are alike, having 23 chromosomes *and an X chromosome. The fertilization* of an ovum, carrying 24 chromosomes—one of which is X—by a spermium with 24 chromosomes—one of which is X—results in the formation of a new individual with 48 chromosomes (including X-X) in its cells, *and* is a *female. In* the case of a spermium *with the* Y chromosome and *an* ovum with its 23 and X chromosomes a male individual with 46 plus X-Y chromosomes in the body cells is produced. It has to be pointed out, however, that this theory is not universally accepted. Two types of mature, human spermia containing either the X or the Y chromosome have not been described morphologically.

The head is pyriform in profile, its anterior margin being compressed into a thin edge. A faint line runs across the head and separates the anterior, paler two thirds from the posterior, darker staining third. The substance of the head is condensed chromatin. It sometimes contains a small clear vacuole. The anterior two thirds of the head are covered by the *head cap*, a very thin, tightly adhering, structureless membrane; its posterior edge causes the transverse line just *mentioned*.

The transition between the head and the middle piece is the *neck*. It consists of the darkly staining anterior knob, a pale, intermediate, flexible mass and the posterior knob. The fibrillar *axial filament* is attached to the posterior knob and runs uninterruptedly and gradually tapers to the end of the tail. It is surrounded by the inner sheath which is absent from the terminal part of the tail. At the junction of the middle piece and the tail the axial thread with its inner sheath is surrounded by the spiral sheath, and, on the outside, by the spindle-shaped, outer sheath.

Thus, the spermium *contains*, in a *modified* form, all the essential parts of a cell. The nucleus is represented by the head which contains the bulk of the hereditary substance of the male sex cell. The substance of the centrioles is distributed between the anterior and posterior knobs and the terminal ring. When it enters the egg in the process of fertilization, the centrioles of the male cell, and especially the anterior knob, are supposed to furnish to the egg cell the active cytocentrum needed for its cleavage divisions. The idiozome (attraction sphere) and the Golgi body are partly transformed into the acrosome and the head cap. Although most of the cytoplasm is cast off, a small amount of it persists as the outer sheath.

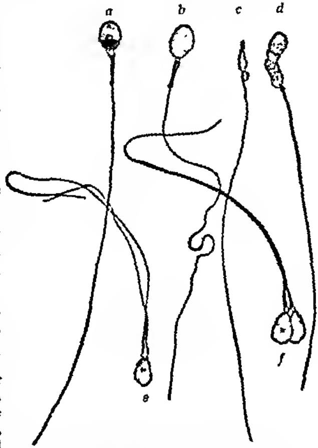

Fig. 451. Human spermia (spermatozoa): *a and b, Head seen from flat surface; c, head seen* in profile; in *c* and *d*, a bit of protoplasm remained attached to the middle piece; e, abnormal spermium with one head and two tails; *f*, abnormal spermium with two heads and one tail. Iron-hematoxylin stain. 1500 ×. (A.A.M.)

The *axial filament of the tail* seems to be the exclusive product of the centrioles. A similar phenomenon is observed in the development of the flagellum from the cytocentrum in the flagellate cells of some invertebrates (sponges) and in the formation of the central flagellum from the centrioles in some epithelial cells of the vertebrates. The inner sheath of the tail, *surrounding* the axial filament, is probably formed by the filament itself.

Spermatogenetic Wave. The successive phases of spermatogenesis are ar-

ranged in the seminiferous tubules according to certain definite rules. The least developed elements are always located nearer the basement membrane, the most developed nearer the lumen. The cause of this movement is purely mechanical and due to growth pressure. The spermatogonia remain adjacent to the basement membrane. The primary spermatocytes, in the early stages of the growth period, may keep this position for a while. The larger cells move toward the lumen and form a second, third, and even fourth layer of cells. The spermatids *form the* inner layer of the epithelium and are usually found in large groups, all the cells of which, originating from a single spermatogonium, show the same stage of development. The same *typical arrangement* in groups is characteristic of the first and second meiotic divisions. The transformations of all cells of one generation occur more or less synchronously.

During the process of spermiogenesis the spermatids seem to require specific external conditions, such as protection and special nutriment. For this purpose they become temporarily closely connected with the Sertoli cells. When the spermia have reached a certain degree of maturity and their cytoplasm has been sloughed off, the whole group leaves the Sertoli cell. This seems to be the result of *mechanical pressure* exerted upon the Sertoli cell body. The new crop of spermatogenic cells, growing around a Sertoli cell, squeezes the spermia out of it in the direction of least resistance. After a short *period of inactivity* the same Sertoli cell receives another, fresh crop of spermatids.

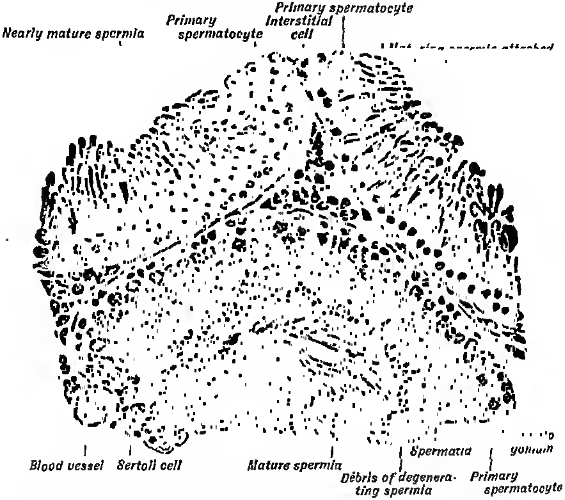

Fig. 452. Testis of rat. The transections of the tubules show various stages of spermatogenesis. 250 ×, reduced to ¾. (A.A.M.)

In the human testis, spermatogenesis, having started at puberty, continues without interruption during the whole period of sexual activity. This also holds true for domesticated mammals, whose sexual activity is not distinctly regulated by the seasons of the year.

The cross sections of the seminiferous tubules show a startling variety of combinations of the different generations of spermatogenic cells. There are, as a rule, four generations in a given section—spermatogonia, spermatocytes (one or both types), spermatids, and spermia. But the degree of maturity of each generation varies in the different combinations. As a rule, a cross section of a tubule shows the same combination of generations along its periphery. In following a *longitudinal section* of an isolated and straightened tubule, one can see, on the contrary, the structure of the epithelium changing continuously. If the position of a certain combination of cells is marked in such a tubule, it will be found that the same combination repeats itself regularly at certain intervals throughout the tubule. *This depends on the fact that spermatogenesis* is a wavelike activity proceeding along the tubule in the direction from the excretory ducts (the tubuli recti) to the periphery of the testis. In the rat the length of the spermatogenetic wave has been found to be 32 mm. The progress of this wave along the tubule is slow. The time necessary for the development of a mature spermium from a spermatogonium is estimated at twenty days for the rat.

In serial cross sections of a tubule, one can see that, in moving away from the tubuli recti, each of the succeeding generations (starting with the mitosis of a spermatogonium) begins somewhat later than its predecessor Besides, the succeeding crops of spermatogenic cells overlap along the tubule because a second, third, and fourth generation start their development before the first generation has reached its full maturity. This explains why a cross section of a tubule always presents four concentrically arranged layers of cells in various conditions of maturity and forming various combinations.

At the stage when the polarity of the male sex cell has been established through the definite attachment of the acrosome and centrioles to opposite ends of the nucleus, groups of spermatids move toward the next Sertoli element, and enter into its cytoplasm. It is possible that this "symphoresis" is regulated by chemotactic influences. The anterior ends of the heads are always directed toward the basement membrane, the growing flagella toward the lumen. Gradually, the heads of the developing spermia may reach deep into the body of the Sertoli cell, almost touching its nucleus. The pyriform, slender cell bodies with the tails are arranged in a bunch and radiate into the lumen. The cytoplasm of the Sertoli cell at this period contains especially numerous fatty and granular inclusions which have been looked upon as food material for the developing spermia.

The diagram (Fig. 453) will help to clarify these complicated conditions. It represents a theoretical cross section of a seminiferous tubule with six sectors, each containing one combination of cell generations; they may be looked upon as six phases of spermatogenesis. Such an ideal cross section does not exist in reality because each tubule in cross section, as a rule, shows the same cell combination along its whole periphery. The six phases have been drawn in one circle to save space.

In the first sector, at the basement membrane, between the inactive Sertoli cells (*S*), five spermatogonia are seen (*Spg*). Centrally to them are five primary spermatocytes (*pSpc*). Toward the *lumen a large group of spermatids (Spt) is* seen. In the second sector some of the spermatogonia of the former stage have developed into additional spermatocytes. The spermatids are connected with the Sertoli cells. In the third sector, at the basement membrane, a young spermatogonium with dustlike nucleus (*Spg"*) is seen. Such cells are present everywhere, but are scattered singly among the older spermatogonia with crustlike nuclei; they could therefore be present in any other sector (*IV, Spg"*). The spermatocytes have grown and are seen moving toward the lumen. In the meantime some new spermatogonia might have been transformed into new primary spermatocytes. The spermiogenesis in the spermatids is progressing. In sector IV the conditions are similar, but the primary spermatocytes have advanced in their growth. In the fifth sector some of the spermatocytes (the younger generation) remain unchanged while others have entered the maturation period. Three mitoses of the first meiotic type are seen (monasters, *pSpc'*); they result in the formation of two resting secondary spermatocytes (*sSpc*). The second meiotic division follows; one dyaster is represented (*sSpc'*). The spermatids have nearly accomplished their transformation. In the sixth sector two spermatogonia are seen in mitosis (*Spg'*). Closer to the lumen, spermatogonia are transformed into a new generation of primary spermatocytes. The mature spermia leave the

Sertoli cells and are cast off into the lumen.

The regular succession of generations of spermatogenic cells and the definite phases of the spermatogenetic wave have been worked out mainly for the testis of the rat, which is a classical object for studies on *spermatogenesis*. In other mammals and especially in man the succession of the generations and the combinations of the different cell types are much less regular. Shorter or longer stretches in the tubules may be in resting condition and not show any spermatogenesis. They alternate with relatively small patches of active epithelium.

In seasonal breeding mammals (for instance, the deer) active spermatogenesis, baving begun for the first time at puberty, is repeated and discontinued periodically for the rest of their lives. Each time it continues only during the period of rut, at the end of which the spermatogenic cells undergo extensive degeneration, and are cast off as débris into the lumen of the tubules Concomitantly, the testis may diminish considerably in size; the seminiferous tubules shrink, show a much smaller diameter and contain only Sertoli cells and some few spermatogonia. In this condition they resemble in structure the tubules of a *prepubertal testis*. *At the beginning of a new period of sexual activity*, the spermatogonia multiply and rapidly produce the various generations of spermatogenic cells, while the Sertoli cells are again compressed and become inconspicuous.

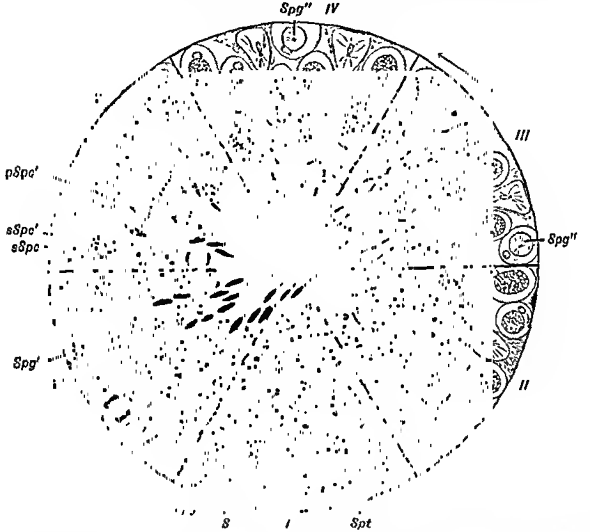

Fig. 453. Diagram of six different stages of spermatogenesis The arrow indicates the succession of stages: *Spg*, Spermatogonium; *S*, Sertoli cell; *pSpc*, primary spermatocyte; *sSpc*, secondary spermatocyte; *Spt*, spermatid. Detailed explanation in the text. Redrawn from Waldeyer.

In the lower vertebrates, these cyclic changes of the testis in connection with the seasons are still more prominent.

Degenerative Phenomena. In the human testis, during the period of spermatogenetic activity, degenerating spermatogenic cells can be found; the spermatids and spermatocytes are most commonly affected. The tubules in a human testis often contain in their lumen masses of degenerating spermatogenic cells which finally disintegrate into granular and fatty detritus. This

need not be considered as a pathologic phenomenon provided it does not exceed certain limits. The degenerating cells are usually seen close to stretches of active seminal epithelium with normal spermatogenesis in full progress.

Very often, abnormal spermatogenic cells can be found. In the spermatogonia this manifests itself usually by an excessive hypertrophy. Among the spermatocytes giant forms are also common, as well as cells with two or more nuclei, *sometimes of unequal size.* They arise through fusion or abnormal mitosis. The spermatids often fuse to form multinucleated giant cells. Spermatids with two or even more nuclei may continue their development and thus monster spermia with two or many tails and sometimes with one tail and two heads may arise.

The degenerating and monster spermatogenic cells are carried, together with the mature spermia, into the epididymis. They disintegrate on their way and their substance is perhaps reabsorbed by the epithelium of the excretory ducts.

The sex cells of the seminiferous epithelium are very sensitive to noxious factors of various kinds. In pathologic conditions of general (infectious diseases, alcoholism, dietary deficiencies) or local (injury, inflammation) character, and under the influence of mental depression, the degenerative changes, and especially the formation of multinucleated giant cells by the coalescing spermatids may become very prominent. Exposure of the testis to a sufficient dose of x-rays causes an extensive degeneration of spermatogenic cells. They are also very sensitive to high temperature. Even the normal temperature of the body of a mammal is incompatible with their normal development. In the majority of the mammals the testes, in the adult, are lodged outside the body in the scrotum which has a lower temperature than the body. It is known that cryptorchid (ectopic) testes, which do not descend into the scrotum, but remain in the abdomen, never produce mature spermia and show atrophic tubules containing Sertoli cells with a few scattered spermatogonia. In experimental cryptorchidism the testis soon shrinks and the tubules collapse and contain only Sertoli cells and remnants of sex cells; after a long time the tubules may disappear (eighteen months in the guinea pig). The seminiferous tubules atrophy in rats fed on a diet lacking vitamin E; they degenerate to a lesser extent in vitamin A deficiency.

In all such cases the Sertoli cells prove to be more resistant than spermatogenic cells. Some few of the spermatogonia, however, seem in most cases to remain intact at the basement membrane between the Sertoli elements. Under favorable conditions, when the noxious factor is removed (for instance, on replacing the artificially ectopic testis in the scrotum), a more or less complete regeneration of the seminiferous epithelium from these residual cells may take place. *In the seminiferous epithelium regenerating after x-ray sterilization, neoformation of spermatogonia from mitotically dividing Sertoli cells has been described.*

Fig. 454. Testis of an eighteen-months cryptorchid guinea pig. Note the groups of interstitial cells, absence of seminiferous tubules. The interstitial connective tissue is slightly edematous. Drawn by Miss A. Nixon from a preparation of C. R. Moore.

In mammals with a very short life, as in the rat, spermatogenesis seems to continue until death without appreciable changes. In man, although spermatogenesis continues far into the senile period, the seminiferous tubules undergo gradual involution with advancing age and the area of active spermatogenesis is more and more reduced. The regressive processes seem to begin very early. A testis of a man older than thirty-five will always show an increasing quantity of irregularly scattered, atrophic tubules, while in the remaining parts of the gland spermatogenesis may continue without visible alterations. Sometimes in very old individuals all the tubules are found shrunken and depleted of spermatogenic cells. The irregular, cleftlike lumen, surrounded by a thickened, hyaline basement membrane, contains only atrophic Sertoli cells

Capsule and the Interstitial Tissue of the Testis. The tunica albuginea, the

mediastinum and the septula of the testis consist of dense connective tissue. The mediastinum contains a few smooth muscle fibers. On the inner surface of the albuginea the dense tissue passes into a looser layer containing many blood vessels—the "tunica vasculosa testis." This layer continues into the interstitial tissue which fills the angular spaces between the convoluted tubules.

The seminiferous epithelium rests on the inner surface of a basement membrane which has a faintly fibrillar structure. Externally the basement membrane is strengthened by a layer of lamellated connective tissue which may be looked upon as a local condensation of the interstitial tissue. The interstitial tissue contains thin collagenous fibers, blood and lymph vessels, nerves, and several types of cells: fibroblasts, fixed macrophages, mast cells, and embryonic perivascular elements. The interstitial cells (of Leydig) which are specific for the testis are also present.

Interstitial Cells. These are scattered in the angular spaces between the tubules in compact groups without a definite relation to the blood vessels. Their body, measuring on the average 14 to 21 μ in diameter, is irregularly polyhedral and is often provided with processes. Transitional forms to much smaller, round or elongated cells are common. The large, spherical or wrinkled nucleus contains coarse chromatin granules, and one or two large nucleoli. Cells with two nuclei are relatively common. Adjacent to the nucleus is a large clear attraction sphere. It contains centrioles which appear as a group of small round granules or as two rod-shaped bodies. The sphere is surrounded by a Golgi apparatus. The peripheral cytoplasm contains numerous mitochondria. The most characteristic features of the interstitial cells are the various inclusions in the cytoplasm outside the sphere.

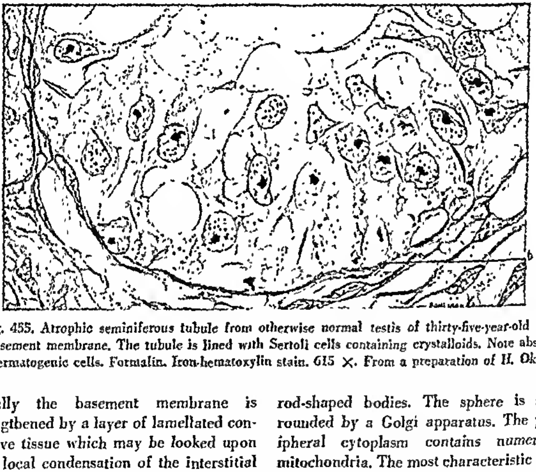

Fig. 455. Atrophic seminiferous tubule from otherwise normal testis of thirty-five-year-old man. *b*, Basement membrane. The tubule is lined with Sertoli cells containing crystalloids. Note absence of spermatogenic cells. Formalin. Iron-hematoxylin stain. 615 ×. From a preparation of H. Okkels.

In fresh condition the cytoplasm is filled with highly refractile granules, many of which react positively to tests for neutral fat and lipoids (sometimes cholesterol esters). Some brownish granules are waste pigment (lipofuscin). The most interesting inclusions are rod-shaped crys-

talloids with rounded or pointed ends (Fig. 456). These are characteristic of the human testis, although they are not of constant occurrence and show great variations in size. Sometimes they seem to dissolve in the peripheral layers of the cytoplasm and their substance leaves the cell body. They are monorefringent, swell in a 10 per cent solution of potassium hydroxide, and are dissolved by hydrochloric acid with pepsin. They are insoluble in 10 per cent hydrochloric, nitric, or acetic acid, and in fat solvents.

It seems that the interstitial cells are modified connective tissue cells. In inflammatory lesions of the testis and in tissue cultures they divide mitotically and become fibroblasts. They may increase in number through transformation of spindle-shaped connective tissue elements, probably of embryonic nature, scattered between the tubules and around the blood vessels.

Some authors claim that the interstitial cells of the testis arise from the same source as the elements of the seminiferous epithelium in the tubules; others believe them to be remnants of the epithelium of the tubules of the mesonephros. Groups of interstitial cells may be found in the connective tissue of the epididymis.

The "epithelioid" character of the interstitial cells suggests the possibility of an endocrine glandular function (see next section).

Endocrine Function of the Testis. The testis, besides producing spermia, causes the development and maintenance of the so-called "secondary sexual characters" and of the sex impulse. In the developing organism it is supposed to regulate the growth of the skeleton and of other parts. After excision of both testes in the prepubertal age, the normal cessation of the growth period of the long bones of the extremities is delayed, and the secondary sexual characters do not develop. If this is done after puberty, the libido gradually disappears, the secondary sexual characters and the auxiliary sex glands (Figs. 465 and 469) undergo partial involution, and disorders of metabolism eventually appear. The implantation of a testis into such an individual may restore normal conditions to a certain extent. In experimental animals the injection of testicular hormone prevents many of these changes from occurring.

Experiments on animals have shown that implantation of a testis may cause the appearance of secondary male characters even in a spayed female. This is due to a hormone secreted by the testis. The chemical composition of the male hormone and its relation to cholesterol are discussed in detail by Koch (1937).

Some authors ascribe the production of this hormone to the interstitial cells; others, to the seminiferous epithelium (spermatogenic and Sertoli cells). A third possibility is, of course, the participation of both elements.

Most of the data favor the first hypothesis. It is known that individuals with cryptorchid testes display, in most cases, a normal sexual behavior and normal secondary characters; they usually retain their virility, although sterility is the rule The seminiferous tubules in the testes of such males are always atrophic, as a result of the higher temperature in the abdomen. In experimental animals with cryptorchid testes of long duration, the seminiferous tubules seem to disappear completely, leaving large masses of interstitial cells. Such individuals, as a rule, keep their libido, the potentia coeundi, and the secondary sexual characters. Similar results were obtained after ligation of the vas deferens or the ductuli efferentes and after large doses of x-rays. Grafts of testicular tissue into castrated animals are supposed to act through their interstitial cells, which proliferate, while the seminiferous tubules become atrophic. These and many other facts indicate that the male

sexual hormone is very probably secreted *by the interstitial cells rather than the* seminiferous epithelium.

The control of hypophyseal activity by the testis is discussed by Moore and Price. The influence of hypophyseal hormones on the testis is discussed in Chapter XIV.

Blood Vessels, Lymphatics, and Nerves of the Testis. The blood supply of the testis is derived mainly from the internal spermatic artery. Some branches penetrate into the interior of the gland in the region of the mediastinum while others run to the anterior side, in or under the albuginea, in the tunica vasculosa. From the mediastinum and from the septula testis, the smaller branches penetrate into the interior of the lobules and break up into capillaries, forming loose networks around the seminiferous tubules. The course of the veins corresponds to that of the arteries. Everywhere in the interstitial tissue between the seminiferous tubules, networks of lymph capillaries with thin endothelial walls can be demonstrated. The nerves, from the plexus spermaticus internus, surround the blood vessels with fine plexuses. The existence of end branches penetrating through the basement membrane into the epithelium of the seminiferous tubules seems doubtful.

EXCRETORY DUCTS

Tubuli Recti and Rete Testis. At the apex of each lobule, its seminiferous tubules join and pass abruptly into the first section of the system of the excretory ducts—*the tubuli recti.* These are short and straight and have a diameter of but 20 to 25 μ. They enter the mediastinum testis and form in its dense connective tissue a system of irregular anastomosing, epithelium-lined, cavernous spaces—the *rete testis* (Fig. 457).

At the transition of the convoluted seminiferous tubules into the tubuli recti the spermatogenic cells in the epithelium disappear and only the Sertoli cells remain. Here they are tall columnar cells, with a cytoplasm containing numerous fat droplets. The cavernous spaces of the rete testis are lined with a cuboidal or squamous epithelium. Its cells are provided with a "central flagellum" and contain fat droplets. No distinct basement membrane is present.

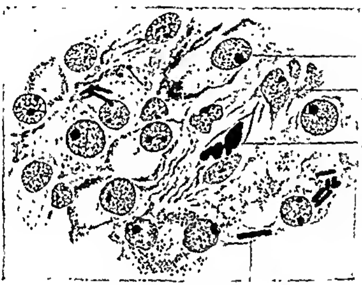

Fig. 456. Human testis from young adult. Groups of interstitial cells with centrioles, darkly stained granular inclusions, and crystalloids. Iron-hematoxylin stain. 650 ×. (A.A.M.)

Ductuli Efferentes. At the upper part of the posterior edge of the testis, 12 to 14 or more efferent ductules arise from *the rete* and emerge on the surface of the testis. They measure about 0.6 mm. in diameter and 4 to 6 cm. in length. Through numerous spiral windings and convolutions they form 5 to 13 conical bodies about 10 mm. in length—*the vascular cones.* These have their bases toward

the free surface of the head of the epididymis and their apices toward the mediastinum testis. They are kept together by connective tissue and constitute part of the head of the epididymis.

The ductuli efferentes possess a very typical epithelium. Their lumen has a festooned outline because it is lined by alternating groups of tall and low cells. The latter form so-called "intra-epithelial glands," small, cuplike excavations in the thickness of the epithelium, not affecting the basement membrane. The low clear cells of these excavations contain pale secretion and pigment granules; there is a brush border and a central flagellum on the free surface; the formation of bleblike outgrowths as a sign of secretory activity has also been described. In animals intravitally stained they contain dye inclusions; this indicates absorption from the lumen. The tall cells usually have a conical form with the broad end toward the lumen. On their free surface are cilia which beat toward the epididymis and move the spermia in this direction. Their cytoplasm stains intensely and contains numerous fat droplets and pigment granules. Very often both cell types are distributed quite irregularly.

Outside of the thin basement membrane, a thin layer of circularly arranged smooth muscle cells can be distinguished. Blood capillaries sometimes invaginate the membrane into the epithelium. In the ducts forming the coni vasculosi, the muscular layer becomes more prominent.

Fig. 457. Rete testis, human; *bv*, blood vessel. 187 ×, reduced to ¾. (A.A.M.)

Ductus Epididymis. The winding tubules of the vascular cones *gradually* fuse into the single ductus epididymidis (Fig. 440). This highly tortuous, long canal (4 to 6 m.) forms, with the surrounding connective tissue, the *body and* the tail of the epididymis. The duct grad-

ually straightens out and merges into the *ductus deferens* which has a length of 40 to 45 cm.

In the proximal, highly convoluted part of the ductus epididymidis, which forms the body of the epididymis, the lumen is lined by a tall, pseudostratified columnar epithelium. The cross sections of the duct have a regular, circular outline. On the inner surface of the basement membrane, small angular basal cells containing lipoid droplets form a discontinuous layer. On their free surface the columnar cells carry a tuft of long (30 μ), nonmotile *stereocilia*, kept together by cytoplasm which plays an important rôle in the discharge of the secretion from the cell body into the canal. In the cytoplasm immediately above the nucleus a Golgi apparatus is present. Nearer to the free surface, a varying quantity of secretion granules, vacuoles, fat droplets, and pigment inclusions are found. They move toward the lumen, leave the cell through the stereocilia, and represent the secretion.

In the distal part of the duct the epithelium gradually becomes lower

The basement membrane is surrounded by a highly developed, capillary network and by a circular layer of smooth muscular fibers which probably help to forward the spermia.

In the proximal part of the duct, in animals stained intravitally with trypan blue, dye granules appear in the cytoplasm. They are supposed to originate in the cells through absorption from the lumen and to move toward the base of the cells. In the epithelium of the epididymis, intra-epithelial, cystlike cavities may develop through vacuolar degeneration of epithelial cells. Occasionally degenerating cells are shed into the lumen.

Nerves (mostly nonmedullated) form a plexus of fine fibers which are connected with the muscles of the vessels and of the wall of the duct: Small sympathetic ganglia have also been described.

A number of rudimentary structures is found attached to the testis and epididymis and to the further sections of the excretory duct. They are remnants of different parts of the embryonic genito-urinary system.

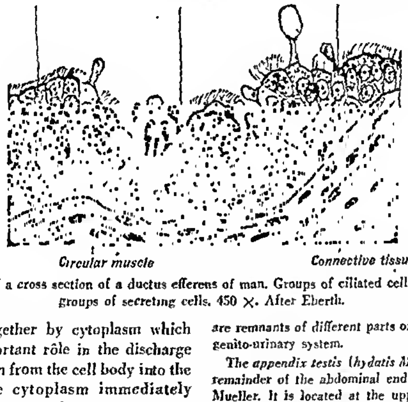

Fig. 458. Part of a cross section of a ductus efferens of man. Groups of ciliated cells alternate with groups of secreting cells. 450 ×. After Eberth.

The *appendix testis* (*hydatis Morgagni*) is the remainder of the abdominal end of the duct of Mueller. It is located at the upper pole of the testis, near the head of the epididymis, as a small nodule consisting of vascular connective tissue and lined with columnar, sometimes ciliated epithelium. The *appendix epididymidis* (pedunculated hydatid) is believed to represent the rudiment of the wolffian body (mesonephros). It is a nodule, 3 by 2 mm., containing a cyst lined with columnar epithelium and connected with the head of the epididymis by a stalk of variable length. The *ductuli aberrantes* are blindly ending epithelial tubules. One of them is found in connection with the rete testis, another with the lower part of the ductus epididymis. The second may sometimes attain the length of 10 or more cm. and forms coils in the connective

tissue of the epididymis. These structures are rudiments of the tubules of the mesonephros. The *paradidymis*, also a rudiment of the wolffian body, is a group of coiled epithelial tubules in the connective tissue of the spermatic cord at the level of the head of the epididymis. In some cases, especially in newborn infants, small nodules with the structure of the cortex of the adrenal may be found in the connective tissue of the tail of the epididymis. In the neighborhood of the paradidymis small accumulations of chromaffin tissue have been described.

face. The connective tissue of the mucous membrane contains extensive elastic networks. The layer of smooth muscles reaches a high grade of development; it consists of an inner, thinner, and an outer, thicker longitudinal layer with a powerful circular, intermediate layer. It attains 1 mm. in thickness and the duct, therefore, is easily palpable through the thin skin of the scrotum. On the periphery, an adventitial coat of connective tissue can be distinguished.

Fig 459. Section of ductus epididymis of a thirty-two-year-old man. The stereocilia are prominent. The lumens contain spermia. Iron-hematoxylin-chromotrop 2 R 72 ×. After Stieve.

Ductus Deferens. On passing into the ductus deferens, the duct develops a larger lumen and a thicker wall. Immediately under the basement membrane a layer of connective tissue can be distinguished as the lamina propria of the mucous membrane. It rises in longitudinal folds, which cause the deeply festooned outlines seen in the cross section (Fig. 461). The epithelium is lower than in the epididymis and has a pseudostratified columnar arrangement with two rows of nuclei. The cells usually have stereocilia on their free sur-

The duct is accompanied by loose, longitudinal strands of smooth muscle—musculus cremaster internus—which form a part of the *spermatic cord*. The other parts of the latter are numerous arteries, convoluted veins of the pampiniform plexus with heavy muscular walls, and nerves of the plexus spermaticus.

The vas deferens, after having crossed the ureter, dilates into a spindle-shaped enlargement, the *ampulla*. At the distal

ually straightens out and merges into the ductus deferens which has a length of 40 to 45 cm.

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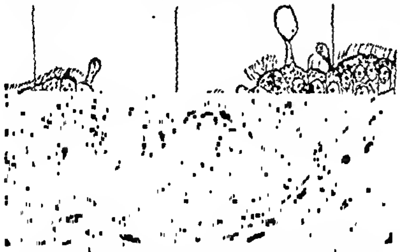


Fig. 458. Part of a cross section of a ductus efferens of man. Groups of ciliated cells alternate with groups of secreting cells. 450 ×. After Eberth.

cilia, kept together by cytoplasm which plays an important rôle in the discharge of the secretion from the cell body into the canal. In the cytoplasm immediately above the nucleus a Golgi apparatus is present. Nearer to the free surface, a varying quantity of secretion granules, vacuoles, fat droplets, and pigment inclusions are found. They move toward the lumen, leave the cell through the stereocilia, and represent the secretion.

In the distal part of the duct the epithelium gradually becomes lower

The basement membrane is surrounded by a highly developed, capillary network and by a circular layer of smooth muscular fibers which probably help to forward the spermia.

are remnants of different parts of the embryonic genito-urinary system.

The *appendix testis* (*hydatis Morgagni*) is the remainder of the abdominal end of the duct of Mueller. It is located at the upper pole of the testis, near the head of the epididymis, as a small nodule consisting of vascular connective tissue and lined with columnar, sometimes ciliated epithelium. The *appendix epididymidis* (*pedunculated hydatid*) is believed to represent the rudiment of the wolffian body (mesonephros). It is a nodule, 3 by 2 mm., containing a cyst lined with columnar epithelium and connected with the head of the epididymis by a stalk of variable length. The *ductuli aberrantes* are blindly ending epithelial tubules. One of them is found in connection with the rete testis, another with the lower part of the ductus epididymis. The second may sometimes attain the length of 10 or more cm. and forms coils in the connective

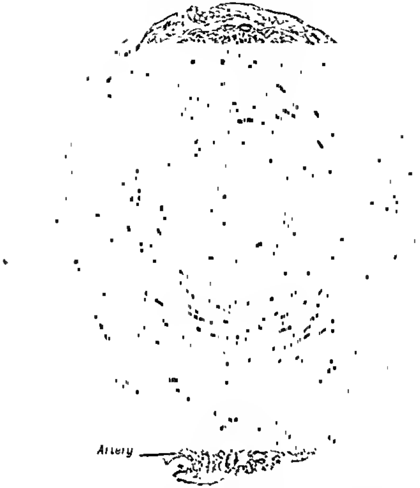


Fig. 461. Ductus deferens of a man, in cross section. 30 ×. After Schaffer.

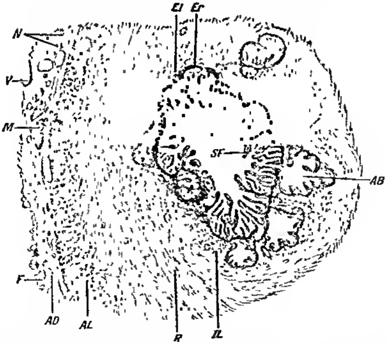


Fig. 462. Portion of a cross section through the ampulla of the ductus deferens of a man: *AB*, Glandlike outpouchings of the main lumen; *L*, Lumen; *AD*, adventitia; *AL*, external longitudinal muscle layer; *El*, longitudinal and, *Er*, ring shaped elastic fibers; *F*, fat tissue; *IL*, internal longitudinal muscle layer; *M*, smooth muscle bundles in the adventitia; *N*, nerves; *R*, circular muscle layer; *SF*, folds of the mucosa; *V*, veins. Orcein stain. 26 ×. After Schaffer.

end of the latter it forms a large, blind, glandular evagination—the *seminal vesicle.* Then, as the short (19 mm.) and straight *ejaculatory* duct (0.3 mm. in diameter), it pierces the body of another gland, the *prostate,* attached to the bottom of the urinary bladder, and opens by a small slit into the prostatic part of the urethra, on a small thickening of its pos-

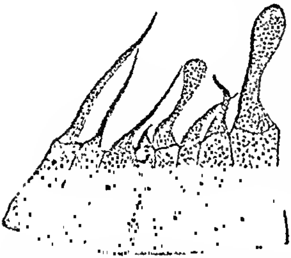


Fig. 460. Section of human ductus epididymis; free surface of epithelium, with stereocilia, discharging secretion. 1000 ×. Redrawn after M. Heidenhain and F. Werner.

terior wall—the *colliculus seminalis* or *verumontanum.* The openings of the ejaculatory ducts are located to the right and to the left of the *utriculus prostaticus,* a blind invagination on the summit of the colliculus.

In the ampulla of the ductus deferens, the mucous membrane with its epithelium is thrown into numerous, thin, irregularly branching folds (Fig. 462, *SF*) which in many places fuse with one another, thus producing in a section a netlike system of partitions with angular meshes. The epithelium may show more or less distinct signs of secretion. From the excavations between the folds, numerous tortuous, branched outpocketings reach far into the surrounding muscular layer (Fig. 462. *AB*) and are lined with a single layer of columnar, clear cells of glandular nature containing secretion granules. The musculature is much less regularly arranged than in the other parts of the ductus deferens.

Ejaculatory Ducts. The epithelium lining the ejaculatory ducts is a simple or pseudostratified, columnar epithelium, probably endowed with glandular functions. Its cells contain a large quantity of yellow pigment granules. Near the opening of the ducts the epithelium often assumes the structure of transitional epithelium. The mucous membrane of the ducts forms many thin folds reaching far into the lumen; its connective tissue is provided with abundant elastic networks. The dorsomedial wall of the ducts contains a series of outpocketings of glandular nature, which are considered by some to be accessory seminal vesicles. The ducts proper are surrounded only by connective tissue.

AUXILIARY GLANDS

The glands associated with the excretory duct of the testis are the seminal vesicles, prostate and bulbo-urethral glands.

Seminal Vesicles. The seminal vesicles are tortuous, elongated, hollow bodies with a very irregular, branched lumen and numerous outpocketings. They are evaginations of the ductus deferens and are similar to it in structure. Their wall consists of an external connective tissue sheet with elastic nets, of a middle layer of smooth muscle thinner than in the duct, and of a mucous membrane resting upon a thin submucous layer. The mucous membrane forms an intricate system of thin, high, primary folds, which branch into secondary and tertiary folds. These project far into the lumen and anastomose very frequently with one another. In this way numerous cavities of different sizes arise; they are separated from one another by thin, branching partitions and all open into the larger central cavity. In sections, however, many of them seem to

ducts, which open independently into the urethra on the right and left side of the colliculus seminalis. The form of the glands is very irregular. Large cavities, sometimes assuming the character of cystic enlargements, alternate with narrow, branching tubules. The blind ends of the upon a layer of connective tissue with dense elastic networks and very numerous blood capillaries. In the larger alveolar cavities it may be low cuboidal or even squamous. In most places it is of a simple or pseudostratified columnar variety. The cytoplasm of the cells contains numerous

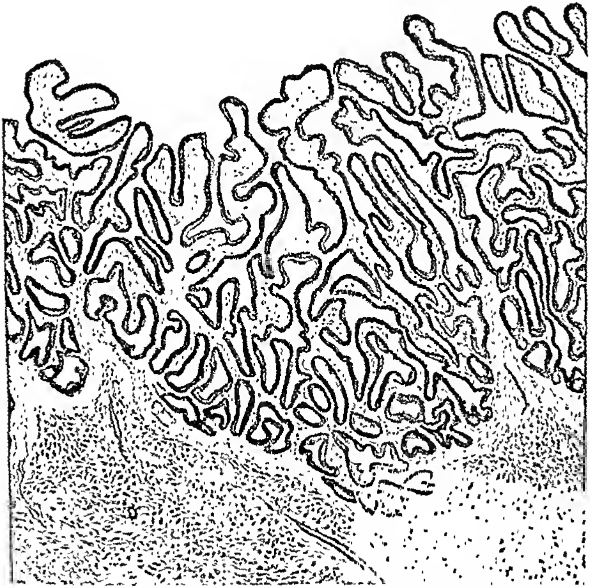


Fig. 464. Section through the wall of human seminal vesicle showing the folded tunica [illegible] e pale-staining lamina propria and the darker muscle coat. 85 ×.

secreting portions are sometimes narrower than the excretory ducts. In many places branching papillae and folds with a thin core of connective tissue project far into the lumen. In sections they may appear as free, epithelium-lined islands in the cavities. There is no distinct basement membrane, and the glandular epithelium rests secretory granules. Some of the latter stain black with iron hematoxylin, but the majority is of lipoid nature. Sometimes, on the free surface of the cells, drops of cytoplasm seem to become detached from the cell body. The epithelial cells become very small and lose their secretion granules after castration. Injections of testicu-

be isolated (Fig. 464). Some are provided with glandlike invaginations similar to those in the ampulla.

The epithelium shows great individual variations, which probably depend on age and on functional influences. As a rule, it is pseudostratified and consists of a layer of round basal cells and of a layer of larger, superficial, cuboidal or low columnar cells. All basal cells possess a pair of centrioles above the nucleus, while in

In many places the epithelial cells, especially in the deeper crypts between the folds and in the glandlike structures, contain secretion granules; on the free surface drops or bleblike formations appear which are cast off into the lumen. The secretion of the seminal vesicles is a yellowish, viscid, sticky liquid containing globulin. In sections it forms coagulated, netlike, deeply staining masses in the lumen. After castration, the epithelium atro-

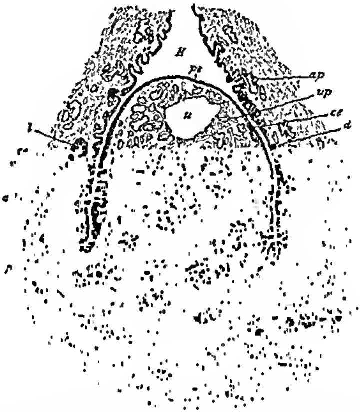


Fig. 463. Cross section through the colliculus seminalis of a young man: Urethra, *H*, incised above; *a*, ejaculatory canal; *ad*, adenoid tissue; *ap*, accessory prostatic gland; *ce*, stratified cylindrical epithelium; *l*, lacuna in cross section; *p*, prostatic ducts; *pe*, stratified epithelium; *up*, prostatic ducts which empty into the utriculus of the prostate, *u*. 10 ×. After v. Ebner, from Schaffer.

the superficial cells the centrioles are located at the surface and form a central flagellum; terminal bars have also been described. The cells contain numerous granules or even large lumps of a yellow pigment; it has a fatty nature, reacts negatively to tests for iron, and makes its first appearance at the time of puberty. A similar pigment is also found in the smooth muscles and in the connective tissue of the seminal vesicles.

phies, but can be restored by injections of testis hormone (Fig. 465).

The muscular wall of the seminal vesicles is provided with a plexus of nerve fibers and with small sympathetic ganglia.

Prostate Gland. The prostate is the size of a horse chestnut and surrounds the urethra at its origin from the urinary bladder. It is a conglomerate of 30 to 50 small, compound tubulo-alveolar glands; they give origin to 16 to 32 excretory

ducts, which open independently into the urethra on the right and left side of the colliculus seminalis. The form of the glands is very irregular. Large cavities, sometimes assuming the character of cystic enlargements, alternate with narrow, branching tubules. The blind ends of the upon a layer of connective tissue with dense elastic networks and very numerous blood capillaries. In the larger alveolar cavities it may be low cuboidal or even squamous. In most places it is of a simple or pseudostratified columnar variety. The cytoplasm of the cells contains numerous

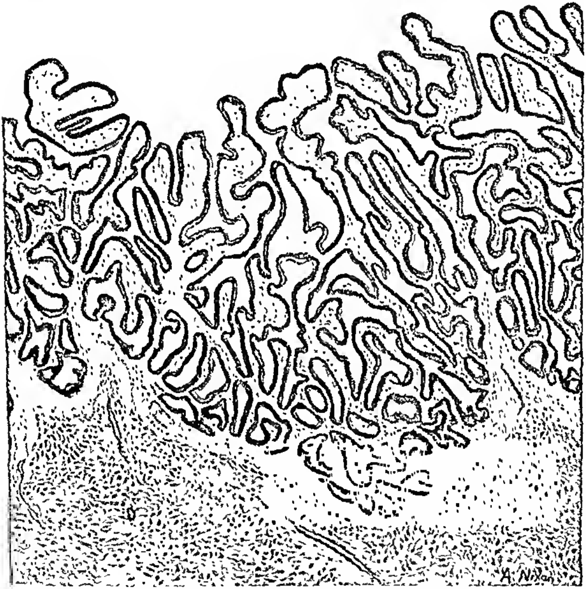
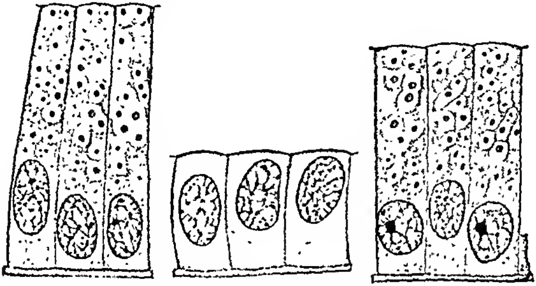


Fig. 464. Section through the wall of human seminal vesicle showing the folded tunica mucosa, the pale-staining lamina propria and the darker muscle coat. 85 ×.

secreting portions are sometimes narrower than the excretory ducts. In many places branching papillae and folds with a thin core of connective tissue project far into the lumen. In sections they may appear as free, epithelium-lined islands in the cavities. There is no distinct basement membrane, and the glandular epithelium rests secretory granules. Some of the latter stain black with iron hematoxylin, but the majority is of lipoid nature. Sometimes, on the free surface of the cells, drops of cytoplasm seem to become detached from the cell body. The epithelial cells become very small and lose their secretion granules after castration. Injections of testicu-

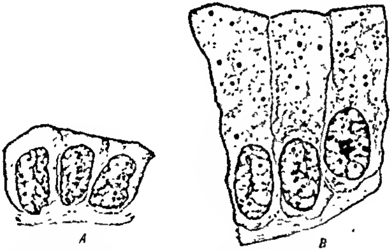
lar hormone restore the cells quickly to their normal appearance and activity (Fig. 469).

The abundant interstitial tissue of the prostate consists of dense connective tissue with collagenous fibers, and elastic networks and many smooth muscles arranged in strands of varying thickness. The connective tissue forms a capsule at the periphery of the organ. Together with the smooth muscles it is arranged in thick, broad septa, widely separating the glands from one another and radiating from the region of the colliculus seminalis to the periphery. Around the urethra the smooth muscles form a thick ring—the internal sphincter of the bladder.



A B C

Fig. 465. Sections of seminal vesicle of rat. *A*, From normal animal; *B*, from twenty-day castrate; *C*, from twenty-day castrate receiving 29 injections of testis hormone in twenty days. Note absence of secretion granules in *B*. Bouin; hematoxylin. Very high magnification. After Moore, Hughes and Gallagher.



A B

Fig. 466. *A*, Atrophic epithelium of seminal vesicle removed from a rat fed on a diet deficient in vitamin B for thirty-two days. *B*, epithelium of other seminal vesicle after the rat had been injected for thirteen days with male hormone. Note the return to normal appearance and compare with Fig. 465. Bouin, hematoxylin preparations of C. R Moore. 2000 ×. Drawn by Miss E. Bohlman.

The *secretion of the prostate* is a thin, opalescent liquid with a slightly alkaline reaction and the odor of semen. The liquid contains proteins, fine lipoid granules in suspension, but no mucus. In sections the secretion in the glandular cavities appears granular. It contains occa-

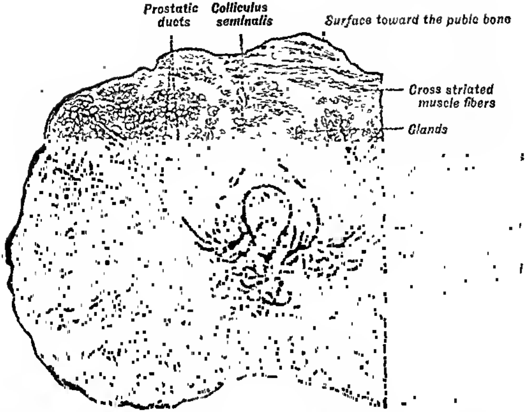


Fig. 467. Low power view of a human prostate. Elastic fibers appear black. 4 ×. After Braus.

Fig. 468. Human prostate: *ep*, Epithelium lining the glandular cavities; *c*, concretions; *m*, smooth muscles in interstitial tissue in longitudinal section; *m'*, same in cross section; *bv*, blood vessels. 250 ×, reduced to ¾. (A A M.)

sional desquamated cells and spherical or oval, often concentrically striated bodies —the *prostatic concretions* (Fig. 468). These originate through condensation of the secretions, may become calcified and exceed 1 mm. in diameter. The concretions are added to the semen and can be found in the ejaculate; the larger ones sometimes remain in the gland and are lodged in cysts. Their number increases with age.

The prostate is abundantly provided with plexuses of mostly nonmyelinated nerve fibers connected with small sympathetic ganglia Sensory nerve endings of various kinds (end bulbs, [illegible]

in the glandular epithelium.

The *utriculus prostaticus*, lodged in the mass of the prostate gland and opening on the colliculus seminalis, according to some recent observations is not merely a vestigial organ without any function, but is an accessory gland of the male sexual apparatus. It is a blind vesicle of considerable size lined by a mucous membrane with many folds and with glandlike invaginations. The epithelium is similar to that of the prostate. Sometimes patches of ciliated columnar epithelium can be found.

Bulbo-urethral Glands. The bulbo-urethral glands (glands of Cowper), each of the size of a pea, are of compound tubulo-alveolar variety and in some respects resemble mucous glands; their ducts enter the posterior section of the cavernous part of the urethra. The ducts as well as the secreting portions are, however, of very irregular size and form, and in many places show cystlike enlargements. The terminal portions end blindly or are connected with one another by anastomoses. *The connective tissue* partitions between the glandular lobules measure 1 to 3 mm. in diameter and contain elastic nets and thick strands of striated and smooth mus-

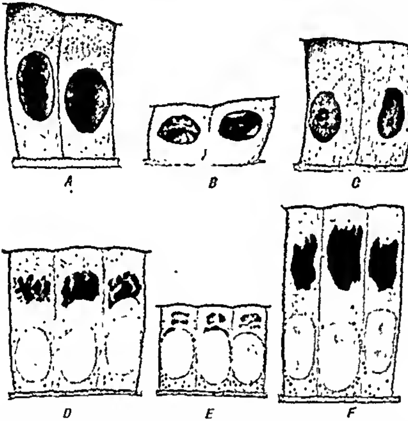


Fig. 469. Sections of the prostate gland of rat. *A, B, C,* From anterior lobe cells; *D, E, F,* from posterior lobe cells; *A, D,* from a normal animal; *B, E,* from a twenty-day castrate; *C, F,* from twenty-day castrate receiving 29 injections of testis hormone in twenty days. The changes in the Golgi net are quite striking in *D, E, F. Mann-Kopsch technic. Very high magnification. After Moore, Price,* and Gallagher

cles. The latter may penetrate with the connective tissue into the interior of the lobules.

The structure of the epithelium in the secreting portions and in the ducts is subject to great functional variations. In the enlarged alveoli the cells are usually flattened; in the other glandular spaces they are cuboidal or columnar with the nuclei at the base. The cytoplasm contains small mucoid droplets and spindle-shaped inclusions staining with acid dyes (Fig. 472). They are supposed to leave the cell body as such and then to dissolve and mix with the mucin. The cells also contain various sized colloidal spherules. The presence of true secretory capillaries is doubtful. The excretory ducts are lined with a pseudostratified epithelium resembling that of the urethra and may contain large patches of secreting cells. Besides, they are provided with small accessory glandular outpocketings having the structure of the glands of Littré of the urethra.

After fixation, the secretion appears in the lumen of the glandular spaces and ducts as angular precipitates which stain brightly with eosin. In life it is a clear, very viscid and lubricant, mucus-like substance which can be drawn out easily into long thin threads. Unlike true mucus it does not form a precipitate with acetic acid.

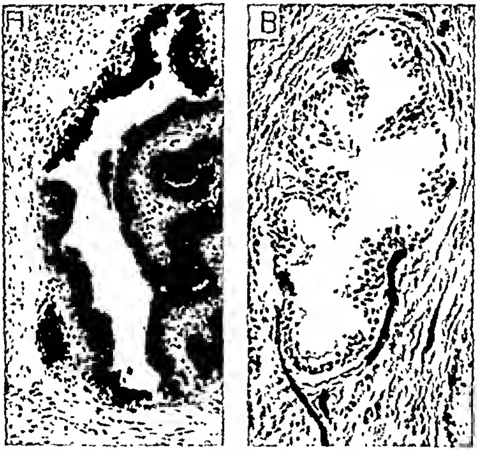
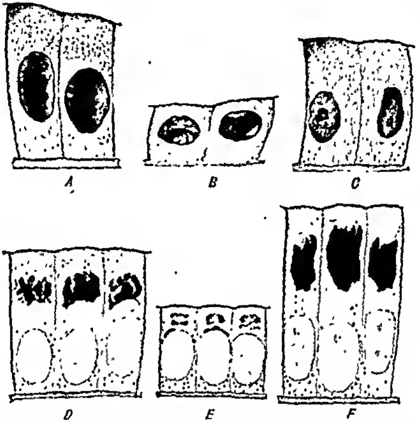


Fig. 470. Photomicrographs of sections of human prostate. *A*, Black stained acid phosphatase obscures the epithelium. *B*, Black stained alkaline phosphatase is limited to the blood vessels. Courtesy of G. Gomori. 180 ×.

THE PENIS

The penis is formed by three cylindrical bodies of cavernous, erectile tissue—the *two corpora cavernosa penis* and the unpaired *corpus cavernosum urethrae* (Fig. 473). The first two are separated from each other in their posterior, divergent parts but join at the pubic angle and run

sional desquamated cells and spherical or oval, often concentrically striated bodies —the *prostatic concretions* (Fig. 468). These originate through condensation of the secretions, may become calcified and exceed 1 mm. in diameter. The concretions are added to the semen and can be found in the ejaculate; the larger ones sometimes remain in the gland and are



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Bulbo-urethral Glands. The bulbo-urethral glands (glands of Cowper), each of the size of a pea, are of compound tubulo-alveolar variety and in some respects resemble mucous glands; their ducts enter the posterior section of the cavernous part of the urethra. The ducts as well as the secreting portions are, however, of very irregular size and form, and in many places show cystlike enlargements. The terminal portions end blindly or are connected with one another by anastomoses. The connective tissue partitions between the glandular lobules measure 1 to 3 mm. in diameter and contain elastic nets and thick strands of striated and smooth mus-

occupied by the corpus cavernosum urethrae (spongiosum). The latter, beginning with the bulbus urethrae between the crura of the corpora cavernosa penis, is pierced throughout its length by the *urethra* and ends with a mushroom-shaped enlargement, the *glans penis*, which caps the conical ends of both corpora cavernosa penis.

The erectile tissue of the corpora cavernosa penis is a vast spongelike system of irregular vascular spaces, intercalated between the afferent arteries and the efferent veins. In the relaxed condition of the organ the cavernous spaces contain but little blood and appear as thin irregular clefts (Fig. 474). In erection they are large cavities filled with blood under high pressure. This causes the enlargement and the rigidity of the penis.

Each of the *cavernous bodies* is surrounded by a thick (1 mm.), resistant, fibrous membrane, the *tunica albuginea.* Its collagenous fibers are arranged in an outer, more or less distinctly longitudinal and an inner circular layer, and are accompanied by elastic nets. Between the two cavernous bodies the albuginea forms a fibrous partition, which, especially near the end of the penis, is pierced by numerous transverse clefts through which the cavernous spaces of both sides communicate. On the inner surface of the albuginea, especially in the posterior part of the erectile bodies, there is a layer of dense connective tissue containing a multitude of small veins draining the cavernous spaces.

The cavernous spaces are largest in the central zone of the cavernous bodies. In the collapsed condition, they may have a diameter of 1 mm. Toward the periphery they gradually diminish in size The partitions between them, the *trabeculae,* consist of dense fibrous tissue and contain thick collagenous bundles with fibroblasts, elastic networks, and strands of smooth muscle fibers. Their surface is lined with common endothelium, which continues into that of the arteries and of the veins.

The albuginea of the corpus cavernosum urethrae is much thinner than in the corpora cavernosa penis and contains circularly arranged smooth muscle fibers in its inner layer. It also is provided with abundant elastic networks. Unlike those of the corpora cavernosa penis, the blood lacunae here are everywhere the same in size, in the deeper parts as well at the periphery. The trabeculae between them contain more numerous elastic fibers, whereas the smooth muscles are relatively scarce. The cavernous spaces occupying the axis of the corpus cavernosum gradually pass into the venous plexus of the urethral mucosa.

The *glans penis* consists of dense connective tissue containing nets of large anastomosing veins, with circular and longitudinal smooth muscles in their thick walls. The longitudinal muscle strands often bulge into the lumen of the veins.

The skin covering the penis is very thin and is provided with an abundant subcutaneous layer containing smooth muscles but is devoid of fat tissue. The skin has no hairs on the distal part of the penis and only small sweat glands. The glans is covered by a circular fold of the skin, the prepuce. Its inner surface, adjacent to the glans, is moist and has the character of a mucous membrane. On the surface of the glans penis the derma of the skin is fused with the connective tissue between the veins just described. In this region peculiar sebaceous glands are described (*glands of Tyson*), which are not connected with hairs. They show great individual variations in number and distribution.

Blood Vessels. The erectile tissue of the penis is supplied with blood from the arteria penis. It breaks up into several large branches (arteria profunda penis, dorsalis penis, etc.) which run to different parts of the organ, but all anastomose with one another. In all these

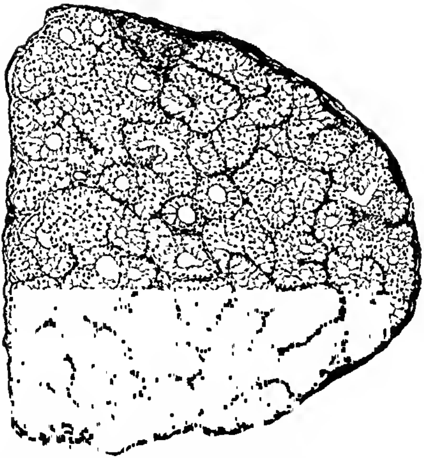

Fig. 471. Lobule of a bulbo-urethral gland of a twenty-three-year-old man. Zenker. 120 ×. Slightly modified after Stieve.

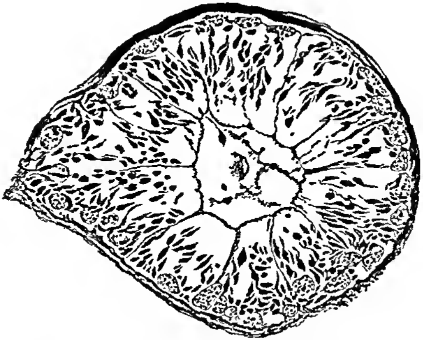

Fig. 472. Alveolus of bulbo-urethral gland (Cowper's gland) of man. Spindle-shaped, darkly stained inclusions in the cells and in the lumen. Mallory stain. 740 ×. Redrawn after Schaffer.

side by side to their pointed ends. On the upper surface of the penis, along the line of their junction, there is a shallow longitudinal groove where the dorsal artery and vein are located. On the lower surface the corpora cavernosa form a deep groove

direct or indirect communication with the largest axial blood spaces

The blood from the corpus spongiosum is drained mainly through the vena dorsalis penis. Unlike those of the corpora cavernosa penis, here the first branches of this vein start from the lacunae with large openings and leave the corpus by the shortest way, by piercing the albuginea.

The arrangement and structure of the afferent and efferent blood vessels in the corpora cavernosa penis explain the *mechanism of erection*. The arteries play the active, the veins the passive, rôle. The erection begins with the relaxation of the tonus of all smooth muscles in the arteries and in the erectile bodies. The blood pressure overpowers the remaining elastic resistance of the tissue, and stretches the media in the arteries. The presence of longitudinal ridges in their intima is believed to enable the lumen in such places to enlarge quickly. The lacunae of the cavernous bodies are filled with arterial blood. As the helicine arteries open especially into the axial, largest spaces, these spaces compress the peripheral, smaller spaces and the thin-walled veins under the albuginea which drain the latter. In this way the outflow of the blood is throttled down, the blood accumulates in the corpora cavernosa under increasing pressure and the erectile tissue becomes rigid. The helicine arteries during erection are passively stretched *out and their convolutions are evened out*. Since in the corpus spongiosum there is no difference between axial and peripheral lacunae and the draining veins are not compressed, there is no noticeable retention of blood and the circulation continues freely. Consequently the corpus spongiosum never attains a great rigidity during erection.

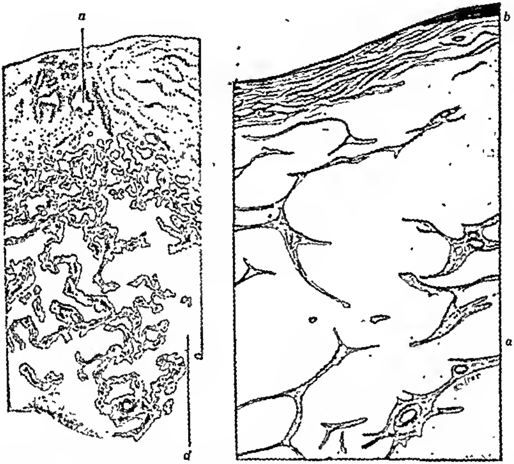

Fig 474. Left: Cross section of crus penis of a man of twenty-seven *a*, Vena profunda penis; *d*, central cavernous spaces Right Crus penis of man; fixed in artificial erection by injection of gelatin. *a*, Central zone; *b*, peripheral zone. Modified and redrawn after Kiss.

After ejaculation the arterial musculature regains its tonus. The afflux of the arterial blood is reduced to the usual degree. The excess of blood, which has accumulated in the corpora cavernosa penis, is slowly pressed out into the veins through the action of the smooth muscles of the trabeculae and through the retraction of the elastic networks. Due to the compression of the peripheral small veins and to the valves described above, the return of the penis into the

branches, even before they enter the erectile tissue, the intima forms, in several places, long ridge-like thickenings, which project into the lumen. They consist of loosely arranged collagenous and elastic fibers, and contain strands of smooth muscle fibers, mostly arranged longitudinally.

Wherever the arterial branches enter into the corpora cavernosa through the albuginea, they assume a longitudinal, forward course, and give off many new branches. The larger branches form the majority, their special purpose is to fill the cavernous spaces for the erection. In the quiescent condition of the penis they have a convoluted or curled course—*helicine arteries.* They have a very thick media. When they reach 65 to 80 μ in diameter (precavernous arteries) they run in the longitudinal trabeculae of the corpora cavernosa, and open directly into the cavernous spaces without forming capillaries

The intima of the helicine arteries is also provided with longitudinal ridges of connective tissue and smooth muscles, as has been described for the branches of the arteria penis before they enter the erectile tissue. The ridges are located especially at the places of division of the vessel. The generally admitted existence of short, cushion-like pads projecting far into the lumen and acting as valves, has not been confirmed by recent investigations.

The arterial supply of the corpus spongiosum is similar to that of the corpora cavernosa penis.

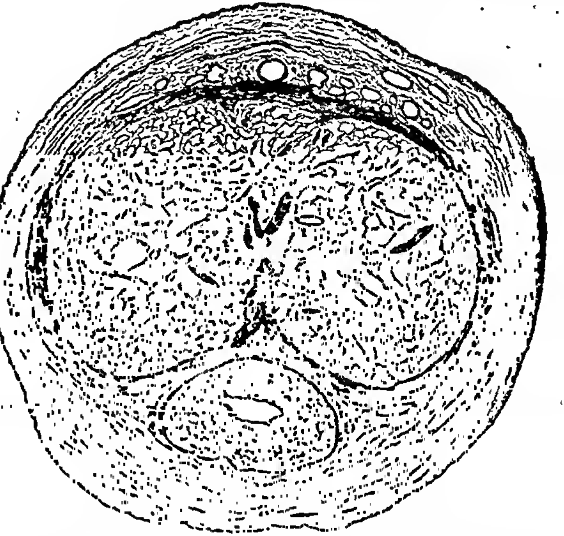

Fig. 473. Cross section of the penis of a twenty-one-year-old man. The septum in the corpus cavernosum penis is incomplete as the section is from the distal part of the organ. The penis was fixed by the injection of formalin into the corpus cavernosum. 3½ ×. Slightly modified after Stieve.

The major part of the blood leaves the corpora cavernosa penis through the vena profunda penis. Its radicles have a thick muscular wall. They arise under the albuginea, especially in the posterior regions of the erectile bodies, through confluence of a multitude of branched "post-cavernous" venules. The latter run parallel to the surface under the albuginea, have a length of 300 to 400 μ or more, and do not possess any muscles in their thin walls. They originate from the peripheral cavernous spaces which are in

direct or indirect communication with the largest axial blood spaces.

The blood from the corpus spongiosum is drained mainly through the vena dorsalis penis. Unlike those of the corpora cavernosa penis, here the first branches of this vein start from the lacunae with large openings and leave the corpus by the shortest way, by piercing the albuginea.

The arrangement and structure of the afferent and efferent blood vessels in the corpora cavernosa penis explain the *mechanism of erection* The arteries play the active, the veins the passive, rôle The erection begins with the relaxation of the tonus of all smooth muscles in the arteries and in the erectile bodies The blood pressure overpowers the remaining elastic resistance of the tissue, and stretches the media in the arteries The presence of longitudinal ridges in their intima is believed to enable the lumen in such places to enlarge quickly. The lacunae of the cavernous bodies are filled with arterial blood. As the helicine arteries open especially into the axial, largest spaces, these spaces compress the peripheral, smaller spaces and the thin-walled veins under the albuginea which drain the latter. In this way the outflow of the blood is throttled down, the blood accumulates in the corpora cavernosa under increasing pressure and the erectile tissue becomes rigid. The helicine arteries during erection are passively stretched out and their convolutions are evened out. Since in the corpus spongiosum there is no difference between axial and peripheral lacunae and the draining veins are not compressed, there is no noticeable retention of blood and the circulation continues freely. Consequently the corpus spongiosum never attains a great rigidity during erection.

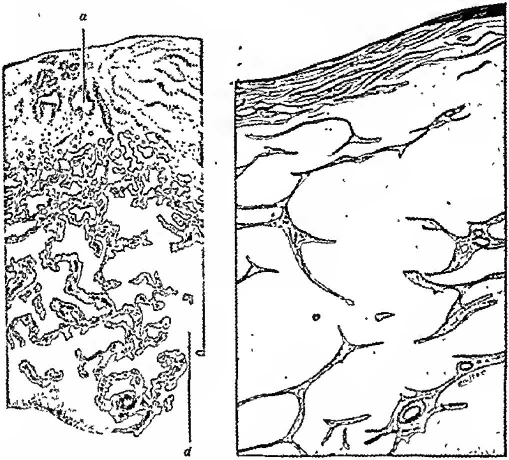

Fig. 474. Left Cross section of crus penis of a man of twenty-seven *a*, Vena profunda penis; *d*, central cavernous spaces. Right Crus penis of man; fixed in artificial erection by injection of gelatin. *a*, Central zone; *b*, peripheral zone. Modified and redrawn after Kiss.

After ejaculation the arterial musculature regains its tonus. The afflux of the arterial blood is reduced to the usual degree. The excess of blood, which has accumulated in the corpora cavernosa penis, is slowly pressed out into the veins through the action of the smooth muscles of the trabeculae and through the retraction of the elastic networks. Due to the compression of the peripheral small veins and to the valves described above, the return of the penis into the

flaccid condition is only accomplished gradually.

Lymphatics. Dense, superficial networks of lymphatic capillaries are found in the skin of the penis, of the prepuce and of the shaft. They form a dorsal superficial lymph vessel, which runs toward the medial inguinal lymph nodes. Deep nets of lymphatic capillaries collect the lymph from the glans; they form a plexus on each side of the frenulum and continue into a dorsal subfascial lymph vessel.

Nerves. The nerves of the penis belong to the cerebrospinal (nervi pudendi) and to the sympathetic (plexus cavernosus) systems. They first supply the striated muscles of the penis (such as the bulbocavernosus) and also furnish the sensory nerve endings in the skin and the mucous membrane of the urethra. Among these sensory endings, free branching nerve endings in the epithelium of the glans, the prepuce and the urethra can be distinguished. Besides, there are free nerve endings in the subepithelial connective tissue of the skin and the urethra. Thirdly, numerous encapsulated corpuscles of various types are present: Corpuscles of Meissner in the papillae of the skin of the prepuce and the glans, genital corpuscles in the deeper layers of the stratum papillare of the derma of the glans and in the mucous membrane of the urethra, and corpuscles of Vater-Pacini along the dorsal vein in the subcutaneous fascia, in the deeper connective tissue of the glans and under the albuginea in the corpora cavernosa. The sympathetic nervous plexuses are connected with the smooth muscles of the vessels and form extensive, nonmyelinated networks in the smooth muscles of the trabeculae in the corpora cavernosa.

SEMEN

As the spermia pass along the excretory ducts, the secretions of the ducts and accessory glands are added to them; the final product is the semen.

The spermia in the seminiferous tubules seem to be nonmotile. They are slowly forwarded into the tubuli recti and the rete testis with the small quantity of liquid in which they are suspended. The moving force is perhaps the passive pressure of liquid accumulating in the tubules which cannot expand because they are surrounded by the firm albuginea. In the ductuli efferentes, the epithelium with cilia beating toward the epididymis takes care of the further transportation of the spermia. The glandular cells devoid of cilia undoubtedly add their secretion to the moving mass.

The long, winding duct of the epididymis is slowly transversed by the spermia. They are kept here, especially in the tail, for a long time, sometimes for months. Here the majority of them lose the last remnant of cytoplasm attached to the middle piece.

What forces are instrumental in their forward motion in the canal is not quite clear. Capillary forces may play a rôle, and a part of the way seems to be made through the movements of the spermia themselves. During ejaculation the contractions of the circular smooth muscles surrounding the tubules must of course be of primary importance.

The thick and viscid secretion of the epithelium of the ductus epididymidis adds nutritive material to the spermia. As a rule, spermia taken from the epididymis are more resistant to environmental changes than those from the testis.

The spermia do not accumulate in the ductus deferens. This part of the excretory system with its heavy muscular coat is adapted only to their speedy transportation.

The function of the seminal vesicles seems to be primarily glandular; their thick secretion is added, during ejaculation, to the mass of the spermia, which pass through the vas deferens and the ampulla into the ejaculatory ducts.

In the process of *ejaculation* the muscular tissue of the prostate also contracts and discharges its abundant liquid secretion; it dilutes the thick part of the semen and stimulates the movements of the spermia. The semen, entering the urethra and mixing with the secretion of the glands of Cowper and Littré is thrown out through the contraction of the bulbocavernosus muscle compressing the bulbus urethrae.

The spermia are believed to number about 60,000 in a cubic millimeter of semen; each ejaculate on the average contains 200 to 300 millions swiftly moving spermia. The tail performs whipping, undulatory movements; the spermium, therefore, advances with the head forward and simultaneously rotates around its long axis. Its speed is considerable—14 to 23 μ in a second.

The spermia are highly resistant elements. Under suitable conditions they may remain alive outside the body for several days and also in the excretory ducts after death. In the uterus and the fallopian tube, living spermia have been found some days after coitus.

Besides the spermia, the semen contains degenerated cells, probably cast off from the epithelium of the excretory ducts and the urethra. Occasionally, columnar epithelial cells and wandering cells of connective tissue origin may also occur. There are, furthermore, round, hyaline bodies of unknown origin, lipoid granules, at times concretions from the prostate and a multitude of fat, protein, and pigment granules. When

the semen cools and begins to dry, peculiar crystals of various forms develop—the *spermia crystals of Böttcher*. They are believed to consist of phosphate of spermin

It has been claimed that the different components of the semen are discharged from the urethra in a certain sequence. With the development of the erection the slimy secretion of the glands of Cowper and Littré lubricates the urethra. At the beginning of the ejaculation the prostatic secretion is discharged first. Being alkaline, it neutralizes the acid reaction of the urethra, where remnants of urine may be present, and the equally acid reaction of the vaginal mucus. Then the masses of spermia accumulated in the vas deferens and the ductus epididymidis are thrown out The final portion of the ejaculate is probably represented by the thick, alkaline, globulin-containing secretion of the seminal vesicles. In some animals (mouse) the abundant secretion of the seminal vesicles is coagulated in the vagina by an enzyme contained in the prostatic juice and thus a solid plug is formed in the vagina which temporarily occludes its lumen and prevents the backflow of the semen

Histogenesis of the Testis. See p. 583.

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Chapter XXV

FEMALE GENITAL SYSTEM

THE female genital organs consist of the ovaries, a system of excretory ducts (the oviducts, the uterus and vagina), and the external genitalia.

THE OVARY

The human ovary is a slightly flattened bean-shaped body measuring 2.5 to 5 cm. in length, 1.5 to 3 cm. in width and 0.6 to 1.5 cm. in thickness. One of its edges, the *hilus*, is attached by the mesovarium to the broad ligament which extends laterally from the uterus. The free surface of the ovary, although bulging into the peritoneal cavity, is covered by a "germinal epithelium" instead of mesothelium. The ovary undergoes continuous, complex changes during the period of sexual activity. Embedded in its interstitial connective tissue are the *follicles* in which the female sex cells, the *ova*, develop. When the follicles reach maturity they rupture on the surface of the ovary and the ova gain access to the open end of the oviduct.

The thick peripheral layer or cortex of the ovary contains the follicles and surrounds the medulla (zona vasculosa) except at the hilus. The medulla consists of loose connective tissue and a mass of contorted blood vessels which are large in proportion to the size of the ovary. At the hilus strands of smooth muscle fibers extend in from the mesovarium.

Certain vestigial organs are found in connection with the ovary. The most important of them is the *epoophoron*. It consists of several parallel or divergent tubules, located in the mesovarium, running from the hilus of the ovary toward the oviduct and fusing into a longitudinal canal, which is parallel to the oviduct. All these tubules end blindly; they are lined by a low cuboidal or columnar, sometimes ciliated epithelium, and are surrounded by a condensed connective tissue layer containing smooth muscle. The lateral end of the longitudinal duct sometimes ends in a cystlike enlargement—the *hydatid of Morgagni*—while its inner end may extend far toward the uterus as the so-called *duct of Gärtner*. Between the epoophoron and the uterus in the tissue of the broad ligament, especially in the fetus, irregular fragments of epithelial tubules—the *paroophoron*—may be found. The epoophoron is the rudiment of the genital part of the mesonephros and corresponds to the epididymis of the male. The paroophoron is the remnant of the caudal part of the mesonephros and corresponds to the paradidymis in the male.

Germinal Epithelium. The epithelium covering the free surface of the ovary is called the "germinal epithelium," because in the embryo ova appear to arise from it (p. 583). In the infant it is a simple cuboidal or columnar epithelium. In the adult its cells gradually become lower and are sometimes flattened when put under tension. A basement membrane between this epithelium and the underlying connective tissue cannot be detected. In vitally stained animals the cells of the germinal epithelium store masses of dye granules in the infra-nuclear zone. Beneath this germinal epithelium is a layer of dense connective tissue—the *tunica albuginea*.

Follicles. The younger the individual, the more numerous are the follicles. In the newborn infant the follicles in both ovaries were believed to number 400,000. However, a study of serial sections of the

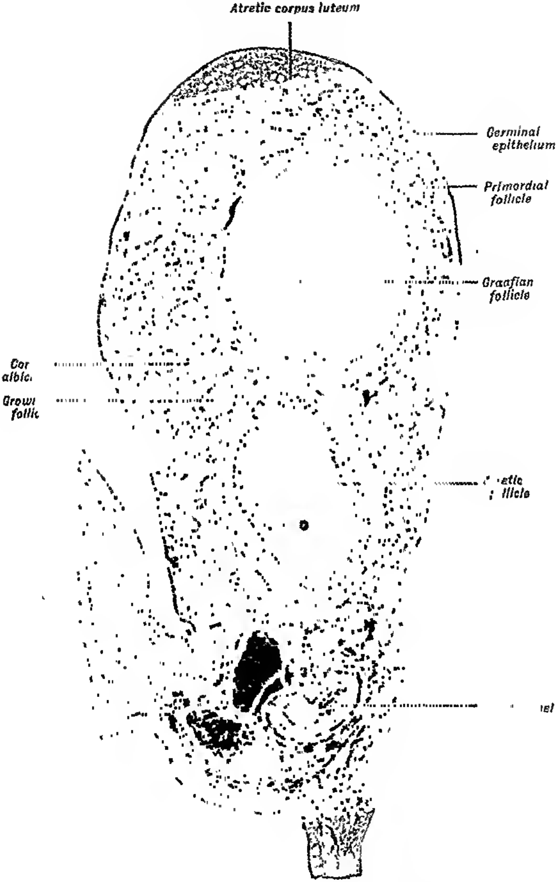


Fig. 475. Transection of the ovary of *Macacus rhesus.* Retouched microphotograph. 42 ×.

ovaries from a normal mature woman of twenty-two years revealed a total of 420,000. Their number decreases progressively throughout life and at the menopause they are hard to find. Most of this decrease is due to *atesia* (pp. 554-556). A few may persist even in old age.

Primary Follicles. The vast majority of the follicles are primary follicles. These are found mainly in the periphery of the cortex and in young individuals they form a thick layer immediately beneath the tunica albuginea. They are probably the source of all the other follicles in primates.

The primary follicles are spheroidal bodies about 45 μ in diameter. The center is occupied by the large round egg cell or ovum. *Its vesicular nucleus* has a slightly eccentric position and contains a loose network of linin threads with small chromatin granules and a large chromatin nucleolus. At that side of the nucleus with the larger amount of cytoplasm, is an accumulation of small mitochondria, the cytocentrum and the Golgi net. The ovum in the primary follicle lacks a membrane; it is separated from the adjacent interstitial tissue by a single layer of flattened follicular cells, 7 to 10 of which appear in a section.

Growing Follicles. The progressive development of a primary follicle consists in growth and changes in the ovum, follicular cells, and the adjacent connective tissue.

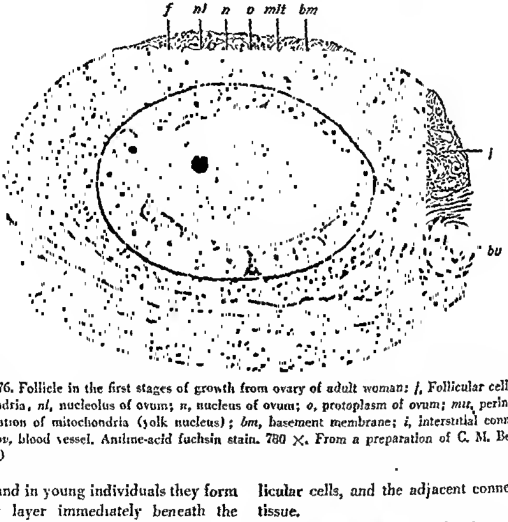


Fig. 476. Follicle in the first stages of growth from ovary of adult woman: *f*, Follicular cells with mitochondria, *nl*, nucleolus of ovum; *n*, nucleus of ovum; *o*, protoplasm of ovum; *mit*, perinuclear accumulation of mitochondria (yolk nucleus); *bm*, basement membrane; *i*, interstitial connective tissue; *bv*, blood vessel. Aniline-acid fuchsin stain. 780 ×. From a preparation of C. M. Bensley. (A.A.M.)

As the egg increases in size, its nucleus enlarges and the mitochondria become more or less evenly distributed in the cytoplasm. Later, yolk granules of two kinds appear (Lewis) (Fig. 481). When the ovum reaches a diameter of 60 to 80 μ, a refractile, deeply staining cell membrane appears. It is called the *zona pellucida* and is probably elaborated by both the ovum and the surrounding follicular cells. It gradually gains in thickness.

The growing follicle enlarges mainly

through the mitotic proliferation of the follicular cells. The few squamous cells of the primary follicle turn first into a layer of columnar cells surrounding the ovum (Fig. 476) and then into a stratified epithelium which thickens more rapidly on one side of the ovum. The follicle assumes an oval form with the *ovum eccentric in* position. Follicles in the deeper zones of the cortex are the first to develop and they expand toward the medulla (Fig. 475).

When the follicle is about 0.2 mm. in diameter, several irregular spaces filled with the clear *liquor folliculi* appear between the follicular cells. The increase in amount of this liquid causes a further increase in the size of the follicle which is now a graafian follicle. In the human ovary, the *separate cavities* usually flow together and the resulting vesicle has a stratified epithelial lining of follicular cells which is thickened on one side. This is the *cumulus oophorus* which surrounds the ovum. The follicular cells have a columnar or polyhedral shape; where the liquor accumulates between them they are angular or stellate and are connected with one another by their processes.

In growing follicles, round darkly staining bodies (of Call-Exner) *surrounded*

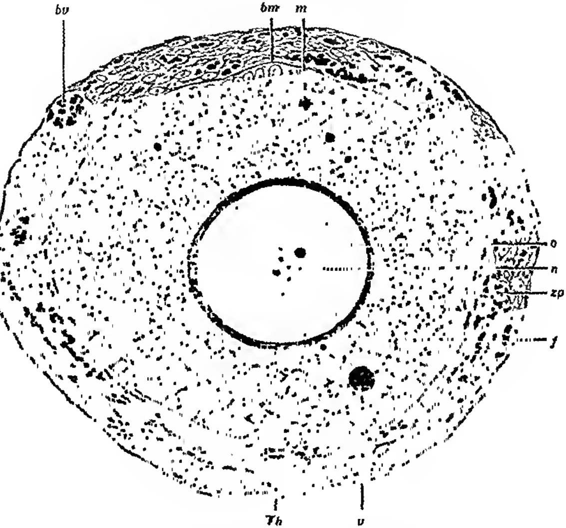


Fig. 477. Growing follicle from human ovary: *o*, Ovum already ⅚ of its full size; *n*, its nucleus; *zp*, zona pellucida; *f*, follicular epithelium with mitochondria, *v*, vacuoles of Call-Exner; *Th*, theca folliculi (outer and inner layer not differentiated as yet); *bv*, blood vessels with erythrocytes; *bm*, basement membrane; *m*, mitosis of follicular cell. 375 ×. (From same section as Fig. 476.) (A.A.M.)

by follicular cells may be found. They probably represent new centers of secretion of follicular liquid. In tissue cultures, the follicular epithelium shows both connective tissue and epithelial characteristics.

A follicle of 0.4 mm. in diameter has a decided polar structure (Fig. 475). The ovum is nearly full grown and is embedded in a solid mass of follicular epithelial cells—the *cumulus oophorus* or *discus proligerus*—which protrudes into the large, liquid-filled cavity. On the other side the *stratified epithelium forms a continuous*, even layer. Meanwhile the connective tissue surrounding the growing follicle differentiates into a capsule, the *theca folliculi*, which is separated from the follicle by a basement membrane.

At first the spindle-shaped cells and reticular fibers are arranged in several concentric layers around the basement membrane. Later this capsule becomes subdivided into two layers. In the *theca interna*, the layer immediately surrounding the basement membrane, an increasing number of blood capillaries develops, and when the follicle has become a vesicle of 2 to 3 mm., the connective tissue cells increase in size and become loosely arranged. The *theca externa* or outer layer keeps its dense structure of concentrically arranged, fusiform cells and thick fibers. As the follicle continues to enlarge, these layers become more and more prominent. There is no sharp limit between the two layers of the theca or between the theca externa and the surrounding stroma.

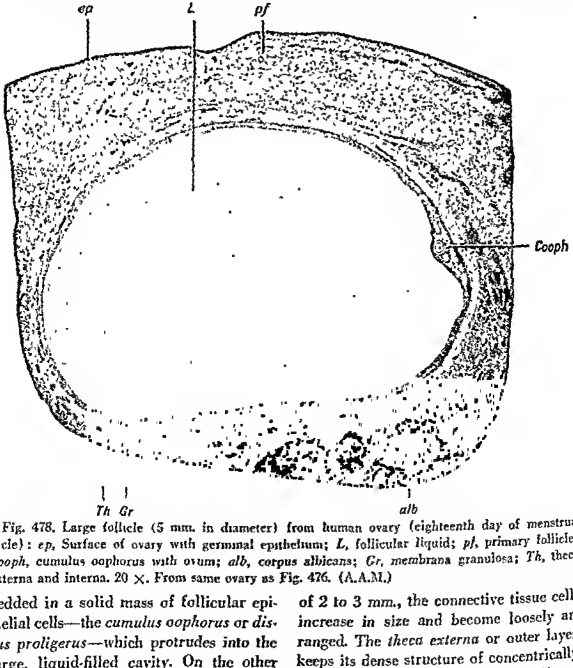


Fig. 478. Large follicle (5 mm. in diameter) from human ovary (eighteenth day of menstrual cycle): *ep*, Surface of ovary with germinal epithelium; *L*, follicular liquid; *pf*, primary follicle; *Cooph*, cumulus oophorus with ovum; *alb*, corpus albicans; *Gr*, membrana granulosa; *Th*, theca externa and interna. 20 ×. From same ovary as Fig. 476. (A.A.M.)

The follicle continues to grow due to the rapid mitotic proliferation of the follicular epithelium and to the progressing accumulation of the follicular liquid. If a follicle is to reach maturity and rupture, it must gradually extend toward the free surface of the ovary. The cause of this is probably the eccentric development of the theca interna, which is thicker and looser on the outer side of the follicle.

or is beginning to involute. In the human ovary a 10-mm. follicle has been found with a normal ovum developing the first polar body.

The protein-containing follicular liquid appears finely granular in fixed sections. The follicular epithelium lining the cavity is often called the *membrana granulosa* and is intimately adherent to the *glassy membrane* which separates it from the connective tissue capsule (*theca*) of the follicle. Between the polyhedral cells of the inner layers of the membrana granulosa, intercellular vacuoles are common. Mitotic figures gradually decrease in number among the granulosa cells in later stages. The connection of the ovum with the membrana granulosa is further loosened by the development of new liquid-filled intercellular spaces in the cumulus.

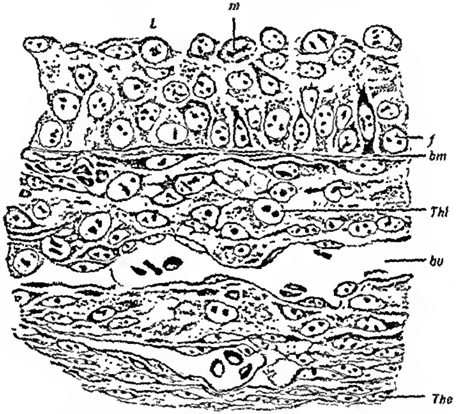


Fig. 479. Part of the wall of the large follicle in Fig. 478, under higher magnification: *f*, Follicular epithelium; *bm*, basement membrane; *Thi*, cells of the theca interna; *bv*, blood vessel; *The*, cells of the theca externa, *m*, mitosis of follicular epithelial cell; *L*, cavity of follicle, all cells contain mitochondria. 780 ×, reduced to ⅘. (A.A.M.)

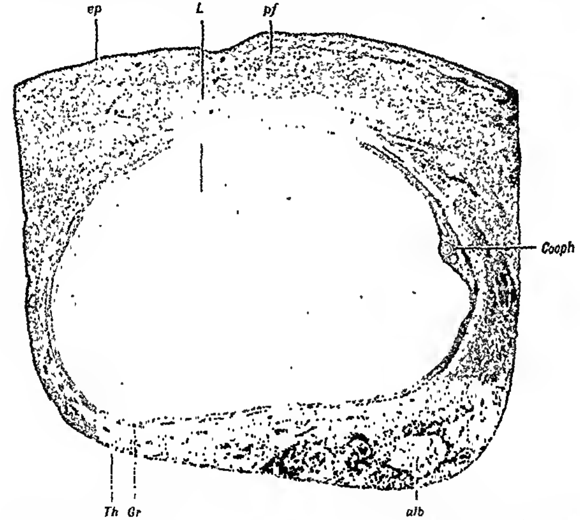
Mature Graafian Follicles. The mature follicles are large vesicles which occupy the thickness of the ovarian cortex and bulge on the free surface of the organ. The liquid in the follicular cavity is under considerable pressure and the outer part of the wall is very thin.

It is believed that a follicle requires about ten to fourteen days to reach maturity. Even with careful study it is somewhat difficult to determine whether a follicle is still growing, has reached maturity,

A layer of columnar follicular cells remains attached to the ovum; near matur-

by follicular cells may be found. They probably represent new centers of secretion of follicular liquid. In tissue cultures, the follicular epithelium shows both connective tissue and epithelial characteristics.

A follicle *of 0.4 mm. in diameter* has a decided polar structure (Fig. 475). The ovum is nearly full grown and is em-



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may degenerate at any stage of development from primordial follicles.

Rupture of Graafian Follicles. Ovulation. The follicular fluid accumulates faster than the follicle grows and so the superficial part of the follicular wall, bulging on the surface of the ovary, becomes progressively thinner. The follicular fluid which forms just before ovulation is more watery than the rest and appears to be secreted at a rapid rate (Robinson). The blood vessels are compressed and the small spot at the apex of the bulging finally opens (Fig. 482).

Through the small opening in the wall of the follicle on the free surface of the ovary, the follicular liquid oozes out into the peritoneal cavity. The ovum, whose connections with the cells of the cumulus oophorus were loosened in the last stages of development, is torn away with the corona radiata from the cumulus and is discharged with the liquid. The immediate cause of the rupture of the follicular wall is not known. In women it usually occurs spontaneously in the intermenstrual period.

This process which frees the ovum and enables it to meet the male sex cell for the purpose of fertilization, is called "ovulation." Each time one ovum is set free, in some cases, two or rarely even more. Sometimes the maturation of a follicle with consequent ovulation occurs alternately in both ovaries, but the same ovary may develop a follicle several times in succession. In the human female a follicle ripens at intervals averaging twenty-eight days, although variations of a week or more are common. Cycles of typical duration not associated with ovulation may occur. It is probable that the fimbriae of the tube closely invest the ovary at the time of ovulation.

Maturation of the Ovum. The ova in the ovary of an adult mammal are in the period of growth and are called *primary ovocytes;* they are homologues of primary spermatocytes. The period of growth in ovogenesis is followed by a period of maturation in which the primary ovocyte undergoes two successive maturation divisions. The resulting four cells have a haploid number of chromosomes. Of these four ova with haploid nuclei, only one develops into the mature ovum. The other three are thrown off as rudimentary structures and degenerate (Fig. 483).

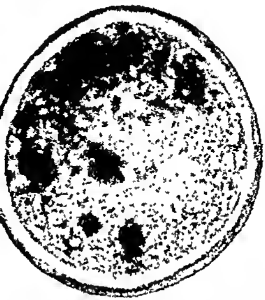


Fig. 481. Photomicrograph of living human ovum. Note yolk granules. The nucleus is not visible. The zona pellucida appears as a bright layer. 450 ×. Courtesy of W. H. Lewis.

The first maturation division begins shortly before ovulation. Although the chromatin is equally divided between the daughter cells, one of them, the secondary ovocyte, receives practically all the cytoplasm of the mother cell; the other becomes the *first polar body*—an abortive secondary ovocyte which degenerates.

Immediately after the expulsion of the first polar body, the nucleus of the secondary ovocyte enters the second meiotic division. The spindle remains at the metaphase and the division is not completed until fertilization. The chromatin mass is divided equally but the bulk of the cytoplasm is again retained by one daughter cell—the mature *ovum* (corresponding to the spermatid). The other daughter cell is the small, abortive *second polar body.* In the human subject incomplete observations on the formation of the polar bodies have been published.

In almost all mammals the first polar body is formed while the ovum is still in the graafian

ity these cells become tall and conspicuous, forming the *corona radiata*, which is believed by some to have protective and nutritive functions similar to those of the Sertoli cells in the testis.

The ovum in the mature follicle reaches a diameter of 120 μ or more. Its surface is immediately surrounded by a thick membrane—the *oolemma* or *zona pellucida.* A "perivitelline space" between the

in the mature ovum. In the living state yolk granules of various sizes and colors are uniformly distributed through the cytoplasm (Fig. 481).

In the matuie follicle the connective tissue capsule reaches its highest development. Its theca externa consists of concentrically arranged fibers and fusiform cells and contains large blood vessels. The theca interna is composed of large poly-

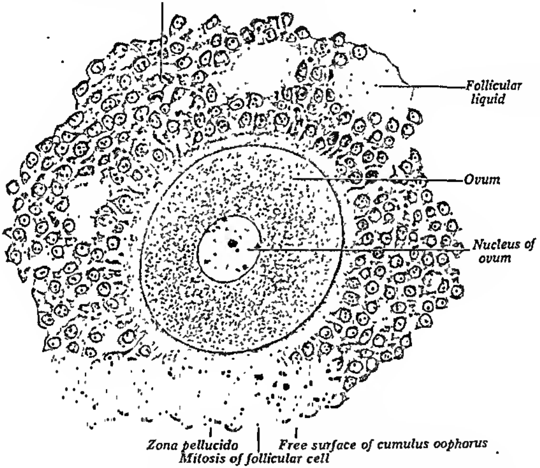


Fig. 480. The ovum of the follicle in Fig. 478 with the surrounding follicular cells under higher magnification. 375 ×. (A.A.M.)

oolemma and the ovum has not been demonstrated in the mammalian egg prior to polar body formation. The cytoplasm of the human ovum contains some yolk granules and in fixed material its peripheral layer is quite clear. The eccentric nucleus (vesicula germinativa) measures 25 μ in diameter, has a thick membrane, a slightly granular linin network, and a large chromatin nucleolus—the *macula germinativa.* A cytocentrum has not been been found

hedral cells with oval nuclei and fine lipoid droplets in their cytoplasm. These are modified connective tissue cells. Between the large cells of the theca interna is a network of thin fibrils continuous with those of the theca externa and the rest of the ovarian stroma. There are many capillaries in the theca interna, close to the basement membrane.

Upon reaching maturity the follicles either rupture or involute, but follicles

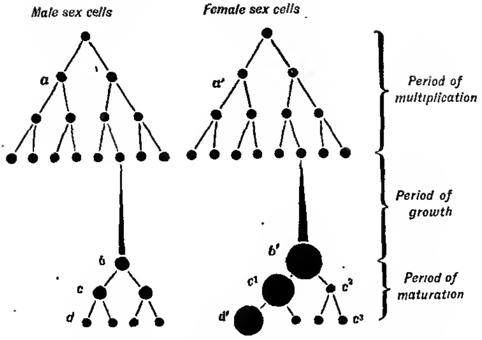


Fig. 483. Diagram of the development of the male (left) and of the female (right) sex cells. Redrawn after Boveri

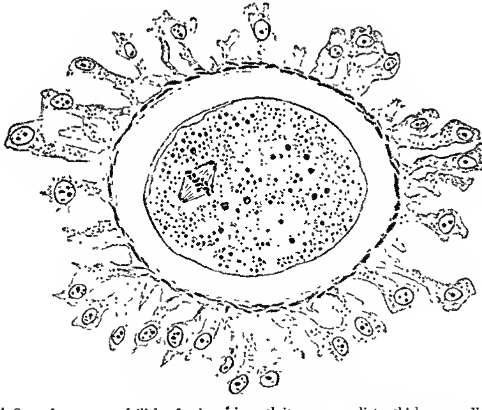


Fig. 484 Ovum from mature follicle of guinea pig, with its corona radiata, thick zona pellucida and the first polar spindle. 530 ×. Redrawn after Rubaschkin

membrane granulosa is thrown into folds and appears considerably thickened. The follicular epithelial cells and the cells of the theca interna change into large pale-staining cells, somewhat like those of the suprarenal cortex. The former follicle is now called the *corpus luteum*. The cavity now has an irregular, stellate shape. The theca externa keeps its regular, ovoid outlines (Fig. 486) while the theca interna,

follicle. The nuclear membrane dissolves and the chromatin material appears as tetrads occupying the equator of an achromatic spindle devoid of centrioles at its apices. Because of their small size and dense grouping it is difficult to count the tetrads. It is certain, however, that their number is one half of the somatic chromosome number of the respective species. The number of tetrads in the first maturation spindle in the human ovum seems to be 24. In the mouse it is 20

The spindle assumes a radial position near the surface of the egg cell and then its outer half, surrounded by a small quantity of cytoplasm, protrudes from the ovum. After metakinesis, this small protoplasmic bud, together with its nuclear material, is pinched off as the first polar body and remains between the ovum and the zona pellucida. It contains 24 dyads. The other 24 dyads remain in the ovum; this nuclear material does not seem to return to the resting stage. The second maturation spindle is formed at once and the 24 dyads are arranged on the equator. The spindle again assumes a radial position at the surface of the egg cell and the second polar body is formed in much the same way as the first. It contains 24 single chromosomes—the haploid number of the human species. The same number of chromosomes remains in the egg cell. They form the egg nucleus and now the ovum is mature.

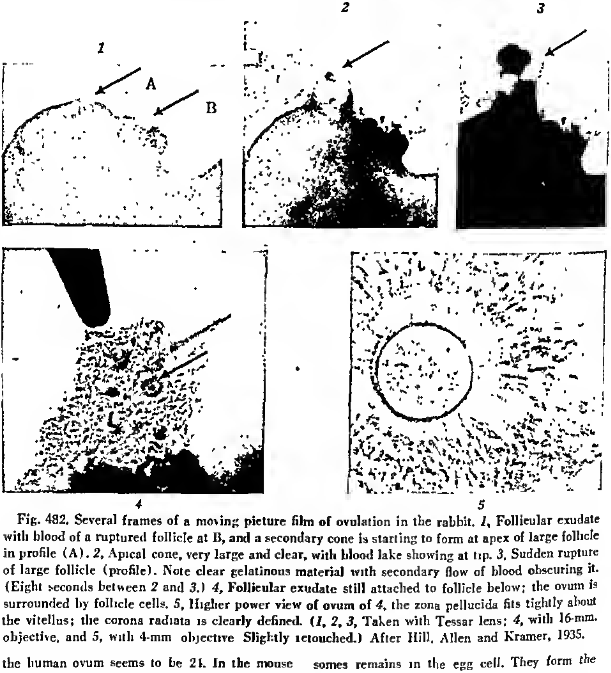


Fig. 482. Several frames of a moving picture film of ovulation in the rabbit. *1*, Follicular exudate with blood of a ruptured follicle at B, and a secondary cone is starting to form at apex of large follicle in profile (A). *2*, Apical cone, very large and clear, with blood lake showing at tip. *3*, Sudden rupture of large follicle (profile). Note clear gelatinous material with secondary flow of blood obscuring it. (Eight seconds between *2* and *3*.) *4*, Follicular exudate still attached to follicle below; the ovum is surrounded by follicle cells. *5*, Higher power view of ovum of *4*, the zona pellucida fits tightly about the vitellus; the corona radiata is clearly defined. (*1*, *2*, *3*, Taken with Tessar lens; *4*, with 16-mm. objective, and *5*, with 4-mm objective Slightly retouched.) After Hill, Allen and Kramer, 1935.

Transformation of the Graafian Follicle after Rupture. The Corpus Luteum. After the rupture of the follicle and the discharge of the liquor and the ovum with its *corona radiata*, the wall of the follicle collapses, and the epithelial

been given the name of *theca lutein cells* (*paralutein cells*). There are no transitions between these two types of lutein cells.

The granulosa lutein cells have a clear, slightly vacuolated cytoplasm, contain a distinct cytocentrum in the vicinity of the nucleus, mitochondria, and a Golgi net. The theca lutein cells are smaller and more highly vacuolated in ordinary sec-

The polyhedral lutein cells are surrounded by a network of sinusoidal blood capillaries with a thin endothelium. They seem to be arranged in radial cords or strands. Between them networks of reticular fibers can be revealed by appropriate methods (Fig. 487).

If the ovum is fertilized, the corpus luteum passes through a period of growth and a period of regression. The corpus

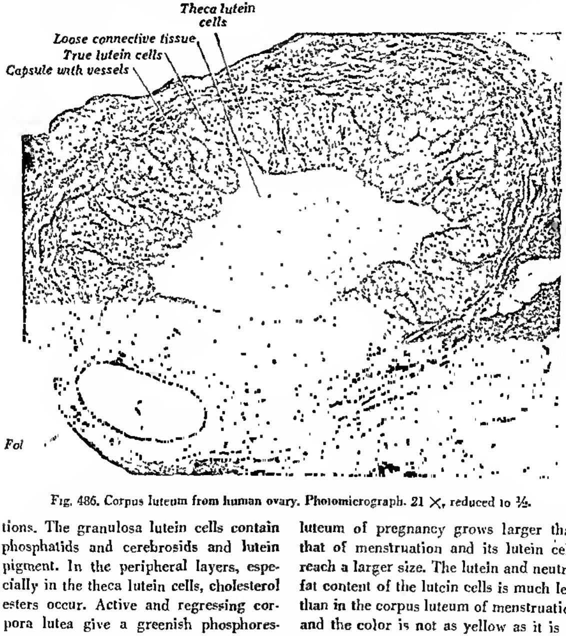


Fig. 486. Corpus luteum from human ovary. Photomicrograph. 21 ×, reduced to ½.

tions. The granulosa lutein cells contain phosphatids and cerebrosids and lutein pigment. In the peripheral layers, especially in the theca lutein cells, cholesterol esters occur. Active and regressing corpora lutea give a greenish phosphorescence in ultraviolet light. According to Popper (1941) this is due to vitamin A. In vitally stained animals the lutein cells may contain granular dye inclusions in regressive stages of their development.

luteum of pregnancy grows larger than that of menstruation and its lutein cells reach a larger size. The lutein and neutral fat content of the lutein cells is much less than in the corpus luteum of menstruation and the color is not as yellow as it is in later stages. Involution of the true corpus luteum usually begins at the fifth or sixth month of gestation. After delivery it proceeds rapidly in qualitatively the same way as in the corpus luteum of menstrua-

on the contrary, loses them. At the base of the folds of the granulosa, the cells of the theca interna accumulate in triangular masses, while between the folds they are scarce or absent.

The details of development of the corpus luteum depend on whether its ovum is fertilized or not. In the former case the corpus luteum becomes a *corpus luteum of pregnancy*, in the latter case a *corpus luteum of menstruation.*

The principal rôle in the formation of the corpus luteum is played by the epithelial follicular cells. They begin at once to hypertrophy and in a few days attain a considerable size. The cell body becomes polyhedral; the nucleus also swells and assumes a spherical form with a coarse chromatin network, and one or two nucleoli. Mitoses may be found, but are rare. Such hypertrophied follicle cells are called *granulosa lutein cells*, although the characteristic lipoid pigment, *lutein*, is at first found only in traces. Simultaneously the spindle-shaped cells of the theca interna, and with them a multitude of capillary sprouts, penetrate radially into the thick layer of follicular cells. When these connective tissue elements reach the inner surface of the folded granulosa layer, they rapidly form a very loose, gelatinous connective tissue, which covers the inner surface of the wall and leaves a space free in the center. This is filled with the remains of the liquor folliculi, transuded serum, and a varying, usually small number of extravasated erythrocytes. The large, lipoid-containing, epithelioid cells of the theca interna remain scattered at the periphery of the folded layer of lutein cells and accumulate in the angle of the folds (Fig. 486). Their aspect and their inner structure are similar to those of the granulosa lutein cells. They have therefore

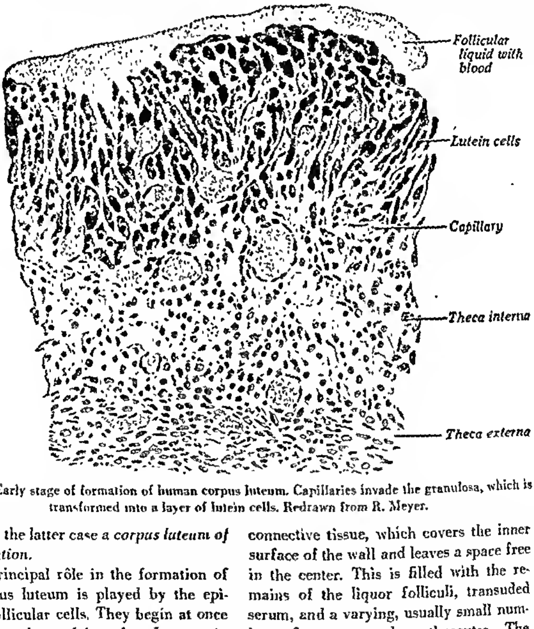


Fig. 485. Early stage of formation of human corpus luteum. Capillaries invade the granulosa, which is transformed into a layer of lutein cells. Redrawn from R. Meyer.

sexual life, and is completed after the menopause. Every normal ovary, therefore, contains degenerating follicles. *Atresia may begin at any stage of development of the follicle*—even in apparently mature ones. It is not known why a few follicles reach maturity and rupture while others degenerate at various stages of development. The ovum always seems to be affected primarily.

If a primary follicle is doomed to destruction, the ovum shrinks and degenerates. The follicular cells may show a tendency to engulf its débris, but they also degenerate quickly, after which the small cavity in the connective tissue stroma is closed without leaving a trace. In the vesicular follicle the ovum and the follicular cells show various signs of degeneration. The connective tissue cells of the theca interna penetrate into the epithelium and absorb it.

With the increase in size of the follicle undergoing atresia, the histologic pictures become more complicated and variable. The first signs of retrogression are always noticed in the egg cell. Its cytoplasm is filled with fat droplets, it becomes coarsely granular and disintegrates. The zona pellucida is highly resistant; it shrinks and collapses, but otherwise seems unchanged. In the substance of the degenerating ovum which has lost it corona radiata and floats freely in the follicular liquid, very often small cells have been observed. They were thought to be cells of the follicular epithelium; it is more probable, however, that they are connective tissue wandering cells which actively penetrate the dead ovum and destroy it.

Before undergoing complete degeneration and disintegration, the ovum in an atretic follicle often shows signs of an atypical, progressive development. Maturation spindles may appear and lead to the formation of more or less characteristic polar bodies. Amitotic fragmentation of the nucleus and division of the protoplasm also occur. These changes result in the appearance in the interior of the zona pellucida of several cell bodies of varying size and with more or less distinct nuclei. These changes have been considered by some authors as possibly being an attempt at parthenogenetic development. They must, of course, be sharply distinguished from cases of ovarian pregnancy in which an ovum retained in a ruptured follicle is fertilized by a spermium finding its way into the ovary.

The follicular epithelium in an atretic follicle always degenerates. The cells first affected are those near the cavity of the follicle. The per-

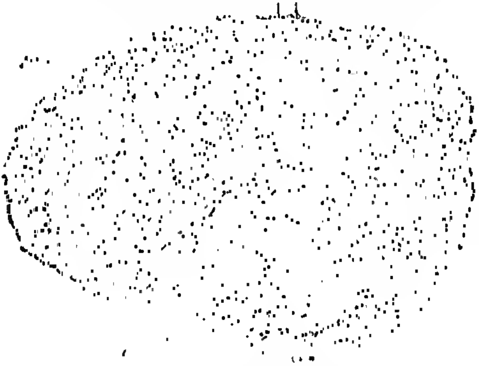


Fig. 488. Corpus atreticum with a well developed theca interna, from the ovary of a thirty-nine-year-old woman 85 ×. After Schaffer.

tion; but, as the corpus luteum of pregnancy is larger, it takes a longer time before *the stage of the corpus albicans* is reached. The final scar is also larger, persists longer, and through its shrinking, usually causes a distinct retraction of the surface of the ovary.

A considerable effusion of blood into the follicle at the moment of ovulation does not occur in the human subject. In the early stages only *an insignificant diapedesis of erythrocytes occurs* in the wall of the collapsed follicle.

After regression has begun the granulosa lutein cells show an increasing infiltration with neutral fat in addition to other lipoids. As the quantity of pigment increases in the human species the lutein border assumes a brighter yellow color. *If there has been* a hemorrhage into the corpus luteum, the connective tissue on the inner surface of the lutein cell layer organizes the blood clot; hemosiderin accumulates in the connective tissue cells; sometimes, extracellular crystals of hematoidin can be found. Later, between the lutein cells, streaks of a hyaline substance appear. The theca lutein cells shrink and gradually disappear.

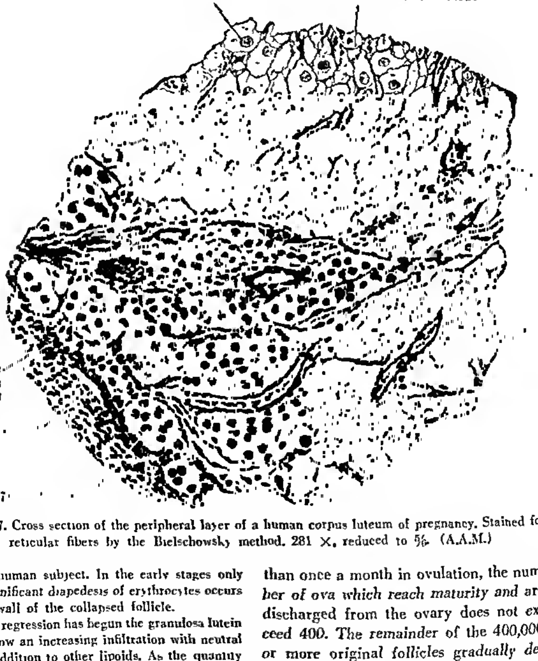


Fig. 487. Cross section of the peripheral layer of a human corpus luteum of pregnancy. Stained for reticular fibers by the Bielschowsky method. 281 ×, reduced to ⅚. (A.A.M.)

Involution (Atresia) of Follicles. As the period of sexual activity in the human female lasts about thirty years and since one ovum is not discharged oftener than once a month in ovulation, the num*ber of ova which reach maturity and* are discharged from the ovary does not exceed 400. The *remainder* of the 400,000 or more original *follicles gradually* degenerate and disappear. This involution of a follicle is called "atresia." It begins in intra-uterine life, becomes very prominent at birth and before puberty, continues on a smaller scale throughout the period of

tex consists of networks of reticular fibers and spindle-shaped cells which resemble smooth muscle cells but do not have fibrils in their cytoplasm. True smooth muscle cells have been described in the theca externa of the follicles of the pig's ovary. The cells of the ovarian stroma are probably not common fibroblasts, for they may give rise to interstitial cells and, in ovarian pregnancy, to decidual cells. Elastic fibers occur in the cortex only in the walls of the blood vessels. Beneath the "germinal" epithelium the interstitial connective tissue is condensed into the tunica albuginea. The peculiar layer of stroma surrounding the follicles, the theca folliculi, has been described above.

The medulla is made of loose connective tissue with many elastic fibers, and accompanying the blood vessels, strands of smooth muscle cells.

Interstitial Cells. Much has been written on the "interstitial cells" of the ovary. In the adult human ovary they are either absent or present in small numbers, as irregular cords of large polyhedral "epitheloid" cells scattered in the stroma. In postembryonic life they arise from the theca interna of atretic follicles. The "interstitial gland" reaches its greatest development in the postembryonic human ovary the first year of life—at a time when atretic follicles are most numerous. It involutes at puberty with the beginning of menstruation and the formation of corpora lutea. During pregnancy and especially at its end, it may increase slightly for a short time.

In the early stages of atresia, the interstitial cells form a layer around the cavity. When this layer is broken up later into separate cell clusters, the stress of growth changes in the stroma scatters the interstitial cells in various directions, and their original relations to the atretic follicles are obscured. In the human ovary they soon degenerate and disappear.

They are best developed in certain animals, for instance in the adult rabbit. Here their cytoplasm contains numerous lipoid droplets which, after being dissolved in fat solvents, cause a typical alveolar structure of the cytoplasm. In contrast to ordinary fat droplets, they are easily dissolved in xylol after blackening with osmic acid. The large, vesicular nucleus has an eccentric position, while the center of the cell body is occupied by a well developed attraction sphere with a group of two or three centrioles. Between the cells a delicate framework of reticular fibers, small spindle-shaped connective tissue cells, and a rich plexus of blood capillaries can be seen. In the adult human ovary they are smaller than in the rabbit and contain cholesterol esters, other lipoids, fat, and, when they degenerate, fatty acids and soaps.

In animals with large litters (rodents) the development of the "interstitial gland," also connected with atresia of follicles, may be enormous (rabbit). The cell foci originating from the breaking up of the hypertrophied theca interna of atretic follicles persist, enlarge, and fuse. Through the continuous addition of new cellular material, therefore, in the adult rabbit, the major part of the organ is transformed into a diffuse mass of large, closely packed, lipoid-containing, interstitial cells, which are structurally almost identical with true granulosa lutein cells. The remaining follicles and the corpora lutea are embedded in this huge cell mass and only a thin albuginea separates the latter from the germinal epithelium on the surface.

In the hilus of the ovary, groups of large, epithelioid cells can be found in intimate connection with bundles of nonmyelinated nerve fibers—the so-called *sympathicotrophic cells.* They are usually considered as chromaffin or pheochrome cells. Their chromaffin nature, however, is not quite certain, since the characteristic brown staining reaction with chromates does not always succeed and since their cytoplasm contains birefringent, lipoid inclusions (cholesterol esters) which are not typical of pheochrome elements.

In the broad ligament and in the mesovarium, small accumulations of "interrenal" tissue (cortex of adrenal) have also been described.

Vessels and Nerves. Relatively large vessels from the anastomosis of ovarian and uterine arteries enter the hilus and, branching profusely, run through the medulla. Because of their tortuous course, they were called arteriae helicinae. As in the corpora cavernosa penis, they may show longitudinal ridges on their intima. In the periphery of the medulla they form a plexus from which smaller twigs penetrate radially between the follicles into the cortex and break up into capillaries. These form dense networks in the theca of the larger follicles at the surface of

ipheral cell adjacent to the basement membrane, as well as the cells of the cumulus, may remain alive for a considerable time and are even said to show mitoses. The retrogressive changes are manifested in chromatolysis of the nucleus and fatty or hyaline degeneration of the cytoplasm. The cells round off, shrink, and float in the liquor folliculi as small, round particles; they contain deeply-staining granules of chromatin.

At a very early stage of atresia, at a time when the follicular epithelium may still seem normal, but undoubtedly is already changed, the connective tissue elements of the theca and blood vessels penetrate the basement membrane in many places and invade the degenerating epithelium. The cavity of the follicle collapses, its outlines, marked by the basement membrane, become wavy, and the cavity is filled by a large number of fibroblasts, wandering cells and blood capillaries. Some of these elements in vitally stained animals accumulate granular dye inclusions. The remnants of the degenerated, follicular epithelium are rapidly resorbed. The folded and collapsed zona pellucida remains alone amid the connective tissue elements.

Simultaneously, the theca interna undergoes important changes (Fig 489, O) The folded basement membrane which separates it from the epithelium often increases in thickness, and is transformed into a layer of hyaline substance. The large cells of the theca interna increase further in size and are usually arranged in radial groups or strands, separated from one another by partitions of smaller, fusiform cells, and fibers (Fig. 488). The cells acquire a typical epithelioid character and are filled with lipoid and fat droplets. They are identical with the theca lutein cells described above, but reach a higher degree of development in the atretic follicle.

The cavity of the atretic follicle, containing the collapsed zona pellucida and connective tissue, is now surrounded by a broad, festooned layer of epithelioid, lipoid-containing theca interna cells, arranged in radial cords and provided with a rich capillary network. The microscopic aspect of such an atretic follicle is similar to an old corpus luteum; therefore, such structures have been called *corpora lutea atretica*. The main differences are, of course, the presence of the degenerated ovum in the center of an atretic follicle and the degenerate granulosa cells.

The ultimate fate of an atretic follicle is shrinkage. Strands of fibrous connective tissue penetrate, together with blood vessels, through the hyaline membrane (Fig. 489, *SM*) into the interior, and compress and destroy the remains of the degenerated elements The resulting scar with its hyaline streaks sometimes resembles a corpus albicans, but is usually much smaller and sooner or later disappears in the stroma of the ovary.

The layer of hypertrophic theca interna cells, which surrounds the cavity of the atretic follicle, is broken up into separate cell islands of various forms and sizes by the invading strands of fibrous tissue. These islands are irregularly scattered in the stroma and may persist for a time. They constitute the so-called "interstitial gland" of the ovary (see following text).

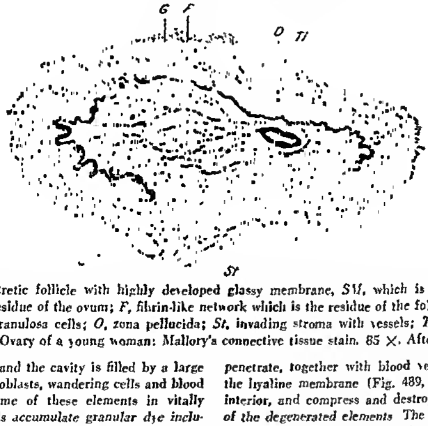


Fig. 489. Atretic follicle with highly developed glassy membrane, *SM*, which is partly broken through. *E*, Residue of the ovum; *F*, fibrin-like network which is the residue of the follicular cavity; *G*, scattered granulosa cells; *O*, zona pellucida; *St*, invading stroma with vessels; *Ti*, cells of the theca interna. Ovary of a young woman: Mallory's connective tissue stain. 85 ×. After Schaffer.

Stroma. The interstitial connective tissue or stroma of the human ovarian cor-

ing, the infundibulum, whose edge is split into many fringes, the fimbriae, the largest of which extends toward the ovary. The ampulla continues into the narrower isthmus adjoining the uterus. The part of the tube traversing the wall of the uterus is the pars interstitialis.

The wall of the oviduct consists of a mucous membrane, a muscular layer and an external serous coat. The mucous membrane in the ampulla is thick and forms numerous, high, branched folds (Fig. 490). In transection the lumen, therefore, looks like a labyrinth of narrow spaces between epithelium-lined partitions. In the pars isthmica the longitudinal folds are much smaller. In the interstitial part they are reduced to low ridges (Fig 492).

The epithelium is of the simple, sometimes pseudostratified, columnar variety. It is highest in the ampulla and diminishes in height toward the uterus. It consists of two kinds of cells. One of these, especially numerous on the fimbriae and ampulla, carries cilia (Fig. 491) which beat toward the uterus. The other is devoid of cilia but is of glandular nature and contains granules. The secretion probably provides the ovum with nutritive material and in some species with an albuminous envelope. In the marsupials a shell as well as albumen are formed about the ova. The two types of epithelial cells are probably merely different functional conditions of one element. In women the epithelium of the oviduct undergoes slight cyclic changes with the uterine mucous membrane (Fig. 491). True glands are absent in the oviduct.

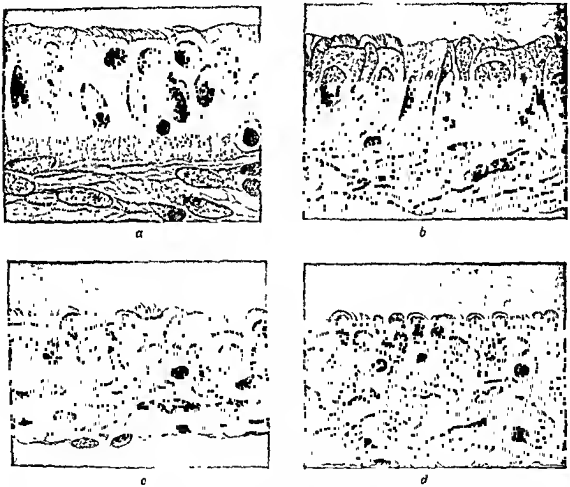


Fig. 491. Epithelium of human fallopian tube showing physiologic changes: *a*, Midinterval; *b*, late interval; *c*, premenstrual; *d*, pregnancy. 700 ×. After Snyder.

The lamina propria (Fig. 492, *M*) of

the basement membrane. In comparison with such capillary nets, that of the *cortex* is very coarse. The veins accompany the arteries; in the medulla they are very large and tortuous and form a plexus in the hilus.

Networks of lymph capillaries arise in the cortex, especially in the theca externa of the large follicles. Lymph vessels with valves are found only outside the hilus.

The nerves of the ovary are derived from the ovarian plexus and from the uterine nerves. They enter the organ through the hilus, together with the blood vessels. They consist for the most part of nonmyelinated fibers; thin myelinated fibers are also present. The presence of sympathetic nerve cells in the ovary has not been confirmed. The majority of the nerves supplies the muscular coat of blood vessels. Many fibers penetrate into the cortex and form plexuses around the follicles and under the germinal epithelium on the surface. It seems doubtful whether they penetrate through the basement membrane into the epithelium of the follicles. Sensory fibers ending in corpuscles of Pacini have been described in the *stroma*.

THE OVIDUCT OR FALLOPIAN TUBE

The oviduct, a muscular tube 1 cm. thick and about 12 cm. long, is attached by the mesosalpinx at the broad ligament to the base and side of the pelvis; it is the proximal part of the müllerian duct of the embryo. Since the uterus arises from the fusion of the two mullerian ducts, the tubal and uterine epithelium are continuous. The myometrial mesenchyme secondarily envelops the uterine end of the tube and so in the adult the tube pierces the fundus of the uterus. The abdominal part of the tube (ampulla) ends in a funnel-shaped open-

Fig. 490. Portion of a cross section through the ampulla of the tube of a twenty-seven-year-old woman: *S*, Mucosa, passing over without a sharp border into the muscular layer; *a*, artery; *f*, folds of the mucous membrane covered with ciliated epithelium and containing blood vessels, *g*; *m*, smooth muscle bundles cut longitudinally and in cross section at *m'*; *v*, veins. 50 ×. After v. Ebner, from Schaffer.

briae, together with the contraction of the muscles, brings the opening of the funnel in close contact with the surface of the ovary.

The rhythmic contractions of the oviduct are probably of primary importance in the transport of the ovum. Contraction waves pass from infundibulum to uterus and the cilia beat in the same direction.

The mucous membrane and its folds as well as the serous coat contain abundant blood and lymph vessels. Larger nerve bundles are found together with the vessels in the serous layer and in the peripheral parts of the longitudinal muscle. The circular muscle layer contains a dense plexus of thin nerve bundles supplying the muscle fibers and penetrating into the mucous membrane.

UTERUS

The uterus is that part of the reproductive passages in which the ovum develops until the time of delivery. In the human subject it is single and represents the parts of the embryonic müllerian ducts which have fused in the midline. Developmental abnormalities range from a deep notch in the fundus to two intact uteri, cervices and vaginae.

The human uterus is a pear-shaped organ flattened in the dorsoventral direction and provided with a correspondingly flattened cavity and a thick muscular wall. Four parts may be distinguished in it: (1) the body (corpus uteri) with its rounded upper end, the fundus; (2) the isthmus—the middle, slightly constricted part; (3) the cervix—the cylindrical lower part with the cervical canal; and (4) the portio vaginalis—the lower end, protruding into the vagina, and pierced by the cervical canal.

The wall of the uterus consists of three layers: the outer—the serous membrane

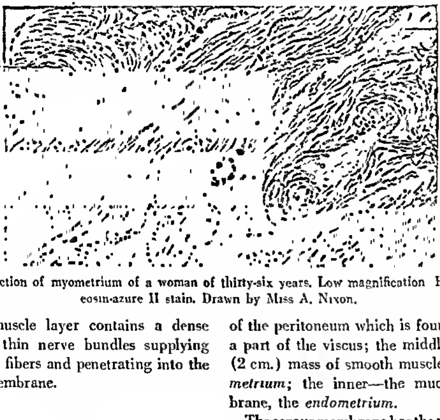


Fig 493. Section of myometrium of a woman of thirty-six years. Low magnification Hematoxylin-eosin-azure II stain. Drawn by Miss A. Nixon.

of the peritoneum which is found only on a part of the viscus; the middle—a thick (2 cm.) mass of smooth muscle, the *myometrium*; the inner—the mucous membrane, the *endometrium*.

The serous membrane has the usual structure of the peritoneum.

Myometrium. The smooth muscle fibers of the muscular layer are arranged in cylindrical or flat bundles separated from one another by interstitial connective tissue containing isolated smooth muscle cells. According to the direction and disposition of the bundles, several layers of muscles can be distinguished in the myometrium, but are not sharply out-

the mucous membrane of the oviduct consists of a network of thin fibers and of numerous fusiform or angular cells. Wandering cells and mast cells also occur. The fixed cells here seem to have the same potencies as in the uterus. In cases of tubal pregnancy, some of them may be transformed into decidual cells.

No true muscularis mucosae and, therefore, no submucous layer can be distinguished. The mucous membrane is immediately surrounded by the muscular coat, which consists of two layers of smooth muscle bundles. The inner layer is circular or spiral, the outer is longitudinal. A distinct limit between the two, however, does not exist because of various spirally directed bundles. Toward the periphery the longitudinal bundles gradually appear in increasing quantities between the circular bundles. They are embedded in an abundant, loose connective tissue with elastic networks and extend far into the serous layer and into the ligamentum latum. Toward the uterus the muscularis increases in thickness. The peritoneal coat of the fallopian tube has the usual serosal structure.

Fig. 492. Portion of the cross section of the fallopian tube of a thirty-nine-year-old woman. Uterine portion (isthmus): *A*, Arteries; *E*, ciliated epithelium; *F*, longitudinal folds of the mucosa in cross section; *L*, longitudinal muscle; *M*, lamina propria; *N*, nerve; *R*, circular muscle; *S*, serosal covering; *V*, veins. 32 ×. After Schaffer.

At the time of ovulation both ovary and oviduct exhibit active movements (Westman). The abdominal opening of the oviduct contains, in its mucosa, a ring of large blood vessels, especially veins, extending into the fimbriae. Between them muscle fibers form a network. It is a sort of erectile tissue. At the time of ovulation the vessels are filled with blood, and the enlargement and turgescence of the fim-

Elastic networks are especially prominent in the peripheral layers of the uterine wall, at the limit between the serosa and the muscularis. From here they extend inward between the muscle bundles. The innermost layers of the myometrium do not contain elastic fibers. The latter are, of course, found everywhere in the wall of the blood vessels. In the cervix the collagenous and elastic elements are especially numerous. This is the cause of the firmer consistency of this part of the uterus.

In pregnancy the connective tissue of the uterus becomes more abundant and succulent, which causes a considerable loosening of coherence between the muscle bundles. In some cases, between the muscle bundles of the myometrium, large accumulations of epithelioid connective tissue cells with fatty and other inclusions may be found. They are presumably of macrophage nature.

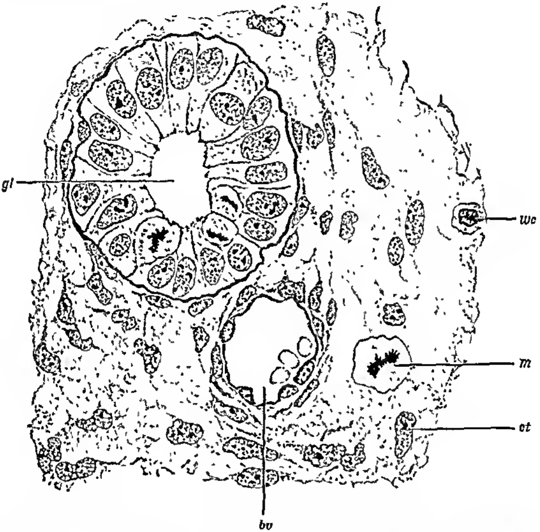


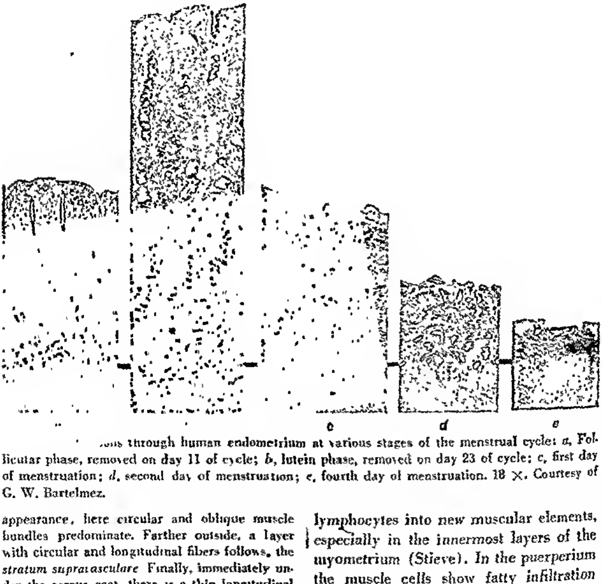
Fig. 495. Mucous membrane of human uterus, eleventh day of menstrual cycle: *gl*, Cross section of gland with mitotically proliferating epithelium, *bv*, blood vessel; *ct*, connective tissue cell of lamina propria; *m*, mitosis of same; *wc*, wandering cell. 500 ×, reduced to ⅚. (From the same mucous membrane as Fig. 494, *a*.) (A.A.M.)

Endometrium. In a sexually mature, nonpregnant woman, the uterine mucosa is subject to cyclic menstrual changes which are closely related to ovarian activity. Beginning with puberty (at the average age of fourteen) and ending with the menopause (usually at the age of forty-five to fifty) every twenty-one to thirty-five days the mucous membrane of the corpus undergoes marked changes.

lined because fibers pass from one layer into another very frequently.

Immediately under the mucous membrane, a thin layer of mostly longitudinal, but some circular and oblique bundles may be distinguished. This is called the ***stratum submucosum.*** *It forms distinct muscular rings around the intramural parts of the oviducts. The next layer to the outside is the thickest; it is called the* ***stratum vasculare,*** *because it contains many large blood vessels, especially veins, which give it a spongy appearance, here circular and oblique muscle bundles predominate. Farther outside, a layer with circular and longitudinal fibers follows, the* ***stratum supravasculare.*** *Finally, immediately under the serous coat, there is a thin longitudinal muscle layer, the* ***stratum subserosum.*** *The two last-named layers send out their muscular bundles into the wall of the oviducts and into the broad and round ligaments.*

The cervix is composed mainly of dense fibrous tissue in which a variable number of smooth muscle cells is distributed at random, according to Danforth.



…ons through human endometrium at various stages of the menstrual cycle: *a*, Follicular phase, removed on day 11 of cycle; *b*, lutein phase, removed on day 23 of cycle; *c*, first day of menstruation; *d*, second day of menstruation; *e*, fourth day of menstruation. 18 ×. Courtesy of G. W. Bartelmez.

The smooth muscle cells of the myometrium have a length of about 50 μ. In the pregnant uterus, when the mass of the organ increases about 24 times, they hypertrophy to a length of more than 500 μ. In this condition there seems also to occur an increase in the number of the muscle fibers through division (Fischer-Wasels) and through transformation of the embryonic connective tissue cells and lymphocytes into new muscular elements, especially in the innermost layers of the myometrium (Stieve). In the puerperium the muscle cells show fatty infiltration and rapidly diminish in size. It is possible that some of them degenerate.

The connective tissue between the muscular bundles consists of collagenous bundles, fibroblasts, embryonic connective tissue cells, macrophages and mast cells. There is a typical reticulum continuous with the collagenous intermuscular tissue.

phages are not uncommon, but for some unknown reason they are not mobilized for phagocytosing extravasated blood. This may perhaps be due to conditions similar to those responsible for the failure of blood to clot after it has been in contact with the endometrial stroma.

The number of uterine glands varies from one individual to another and it may be rapidly increased by budding and growth toward the surface from the basal zone (O'Leary). Such buds which do not reach the surface become dilated (cystic). The glands seem to vary in their proximity to one another during the cycle. This is due largely to changes in the diameter of the glands and in the amount of stromal ground substance (Fig. 494).

The uterine mucous membrane is firmly bound to the underlying myometrium. Occasional strands of endometrium are seen extending down among the muscle bundles. Under pathological conditions the myometrium may be extensively invaded in this manner (adenomyosis). In old age, the endometrium, together with the other parts of the genital system, atrophies and becomes very thin; the glands may become partly obliterated and form small cysts.

Endometrial Blood Supply. Certain arteries pass through the myometrium and basal endometrium with but few branches, to spread out into a rich capillary bed superficially. These vessels are more or less contorted, and are termed "coiled arteries." During most of the cycle they constrict and dilate rhythmically, so that the surface is alternately blanched and suffused with blood (Markee). The basal half of the endometrium is supplied by arterioles with many branches which supply a dense capillary bed in the endometrium and the adjoining myometrium. The zone of endometrium between the superficial and basal circulations is supplied by vessels intermediate in form and has a coarser capillary mesh. The thin-walled veins form an irregular anastomosing net with sinusoidal enlargements at all levels.

Endometrial Cycle. Four phases of activity can be recognized in the endometrial cycle: I. The follicular phase comprises the first half of the cycle and is typically associated with a rapidly growing graafian follicle. II. Progravid (lutein phase), is usually associated with an active corpus luteum. III. Ischemic phase, when little blood flows through the coiled arteries. IV. Menstrual phase, associated with endometrial damage and extravasation.

I. The *follicular phase* usually begins at the end of a menstrual flow and is characterized by numerous mitoses in all endometrial tissues. The glands in the superficial two thirds or more are straight with narrow lumina (Fig. 494, *a*). The secretion accumulates in the glands and their lumina widen as they become wavy in form. Glycogen is present in the gland cells but only a thin mucoid is secreted at this time. The abundant stroma is rich in tissue mucoid. Coiled arteries are not found in the superficial one-third, which has only capillaries and venules.

The phase of hyperplasia may continue for a day or two after ovulation which is believed to occur usually between the tenth and sixteenth days after the onset of a menstrual period. There may be a brief halt in the thickening of the endometrium at this time, a diapedesis of erythrocytes may occur under the surface epithelium, and some blood may occasionally enter the uterine lumen and reach the vagina. This has been termed *intermenstrual bleeding.* According to some it corresponds to the estrous bleeding of the dog.

II. During the *progravid phase* the thickening of the endometrium is due largely to the increase of secretion and edema fluid. The glands become tortuous and then irregularly sacculated, *i. e.*, "pro-

These culminate in its partial destruction, which is accompanied by a more or less abundant extravasation of blood and appears as a bloody vaginal discharge—the *menstrual flow*. The flow, which marks the beginning of the cycle, lasts for three to five days.

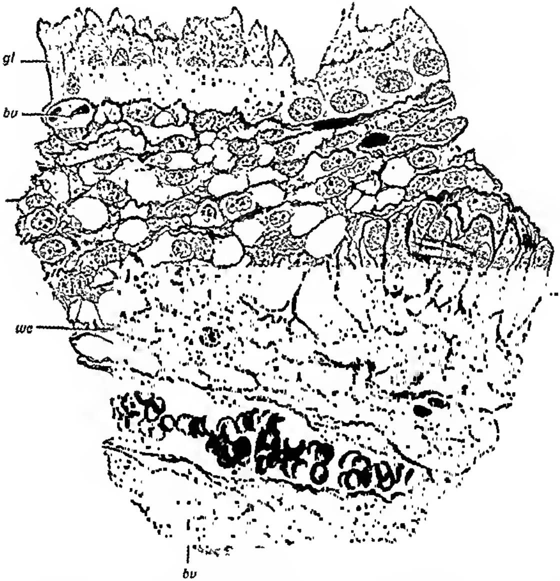


Fig. 496. Mucous membrane of human uterus, twenty-fifth day of menstrual cycle: *gl*, Glandular epithelium; *bv*, blood vessels, *ct*, connective tissue of the lamina propria, with silver impregnated fibrils; *wc*, wandering cell, *art*, artery. 780 ×, reduced to ⅘. (A.A.M.)

The uterus is lined by a simple columnar epithelium. From fundus to vagina small groups of ciliated cells are scattered among the secreting cells. As far as the beginning of the cervical canal this surface epithelium is substantially like that of the *uterine glands* which grow out from it in infancy. These are simple tubules slightly branched in a zone adjacent to the myometrium. They are separated from one another by connective tissue, the *stroma* which resembles mesenchyme. Its irregularly stellate cells have a large ovoid nucleus, the cell processes appear to anastomose throughout the tissue and adhere to the framework of reticular fibers, which are condensed as basement membranes under the epithelium. Elastic fibers are absent except in the walls of the arterioles. There is a ground substance which at times is rich in tissue mucoid; in it are lymphoid wandering cells (Fig. 495, *wc*) and granular leukocytes. Macro-

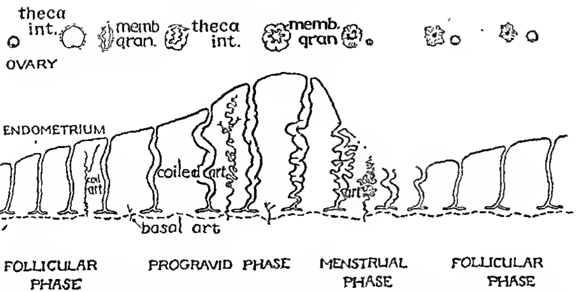


Fig. 497. A diagram of the interrelations of ovary and endometrium in a cycle which involves ovulation. Cycles are said to begin with the onset of menstrual bleeding. One complete cycle and the beginning of another are indicated. The ovarian changes involve the growth of a graafian follicle, its rupture, corpus luteum formation and regression. The endometrial development is indicated by changes in thickness, in the form of the glands and the coiled arteries. These changes depend in part on the time that intervenes between the onset of menstruation and ovulation as well as on the duration of the cycle. Ovulation may occur normally as early as the seventh day or as late as the twenty-second day after the beginning of a menstrual period, but in most instances it probably occurs between the ninth and sixteenth days. The normal variability in cycle length is from twenty-one to forty days. *art.*, coiled arteries; *memb. gran*, membrana granulosa of the graafian follicle and the granulosa lutein cells; *theca int.*, theca interna. Courtesy of G. W. Bartelmez.

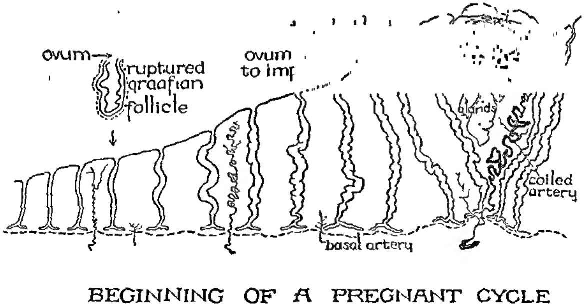


Fig. 498. A diagram showing the beginning of a pregnant cycle. It is identical with Fig. 497 until after implantation, which probably occurs about a week after ovulation. The relations of a recently implanted ovum to the endometrium is from a reconstruction of the ovum described by Brewer and about fourteen days old. It is buried in the endometrium and surrounded by dilated veins which had been opened by the trophoblast. Areas about the ovum with extravasated maternal blood are stippled; two glands below the ovum are dilated with blood. Courtesy of G. W. Bartelmez.

gravid" in form, especially in the middle half of the endometrium (Fig. 494, *b*).

The secretion now contains glycogen and the mucoid is thicker. The gland cells are lower and wider; in the superficial zone they may contain lipoid granules which, however, do not enter the gland lumen. During this phase, the cells readily change their form when immersed in the fixing solution, owing to the presence of much intracellular secretion, and have tongues projecting beyond the terminal bars. (Fig. 496, *gl*) (Bartelmez). In poorly preserved material the cells may even appear continuous with the secretion in the lumen.

In pregnancy the "progravid" changes progress for six to eight weeks (p. 574). In the nonpregnant cycle extensive vascular changes occur thirteen to fourteen days after ovulation and constitute the ischemic phase. This phase was discovered by Markee in transplants of endometrium to the eyes of monkeys.

III. *Ischemic Phase.* A day or more before menstruation the coiled arteries constrict so that the superficial zone is blanched for hours at a time. With this the endometrium shrinks and in the course of two days or more a transplanted bit of endometrium may decrease as much as 76 per cent in area. This involution is due to a loss of secretion and water (edema fluid). The stroma becomes denser. The closely packed stroma cells, irregularly collapsed glands and coiled arteries are characteristic of this phase. Usually many leukocytes are found in the stroma and between the epithelial cells. Sooner or later the coiled arteries clamp down so that the superficial zone becomes anemic, while the blood continues to flow in the more basal portion of the endometrium.

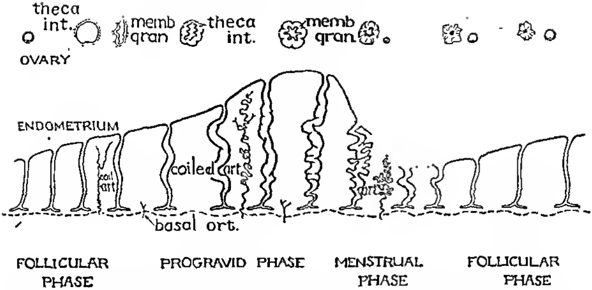
IV. *Menstrual Phase.* After a variable number of hours, the constricted arteries open up for a short time, the walls of vessels near the surface burst, blood pours into the stroma and soon discharges into the uterine lumen. Such blood does not clot. Subsequently patches of blood-soaked tissue separate off, leaving the torn ends of glands and arteries and veins open to the surface. Blood may ooze from such veins, a reflux from the intact basal circulation. The menstrual discharge thus contains altered arterial and venous blood, with normal and hemolyzed, sometimes agglutinated erythrocytes, disintegrated or autolyzed epithelial and stroma cells as well as the secretions of the uterine, the cervical and the vulval glands. Sometimes there are tissue fragments, but blood clots are abnormal. The average loss of blood is 35 cc. By the third or fourth day of the flow the entire uterus may present a raw wound surface. The superficial gland and stroma cells are normal histologically.

Below the zone of extravasation the endometrium remains intact during menstruation, although it does shrink down. Typical progravid glands may be recognizable as such until the end of menstruation (Fig. 494, *d* and *e*). The surviving zone is accordingly wider than the "basalis" of many authors.

Before the vaginal discharge has ceased, epithelial cells glide out from the torn ends of the glands and the surface epithelium is very quickly restored. Then the superficial circulation is resumed, the stroma again becomes succulent and the follicular phase of the new cycle may begin at once.

The "typical" conditions illustrated in Figs. 494 and 497 are not always realized. In fact, the ovary may not produce a ripe follicle in the course of a cycle and then the endometrial changes are minimal. Nevertheless, a clinically typical bleeding occurs at the expected time. This has been termed "anovulatory menstruation."

Such variability introduces difficulties in the evaluation of endometrial tissue removed at operation or biopsy. Errors are also introduced when only small fragments of endometrium are available as in



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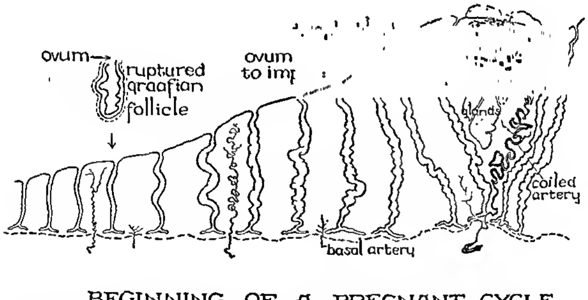

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curettings. The various phases of the cycle in "typical" cases are identified by certain characters: (1) Follicular phase. Endometrium from 1 to 5 mm. thick; straight, narrow glands becoming wavy, the epithelium tall, becoming vacuolated (glycogen), many mitoses in all tissues, no coiled arteries in the superficial one third, and a subepithelial zone free of reticulum postmenstrually. (2) Progravid phase. Endometrium from 2 to 6 mm. thick, glands wavy or sacculated with wide lumen, epithelial cells broad with blebs, stroma edematous superficially, mitoses confined to coiled arteries which are present near the surface. (3) Ischemic phase. Endometrium 3 to 4 mm. thick, greatly contorted arteries and glands, dense stroma with leukocytosis. (4) Menstruation. Endometrium from 0.5 to 3 mm. thick, superficially extravasated blood, the glands and arteries appear collapsed, the stroma is dense and the surface is denuded.

Isthmus and Cervix. The mucous membrane of the corpus passes sometimes gradually, more often abruptly into that of the isthmus which remains thin and shows few signs of cyclic change. It lacks coiled arteries and usually does not bleed during menstruation. It has a dense stroma. The glands are sparse and are oblique to the surface.

The mucosa of the cervix has a different structure. It has a firmer consistency, a thickness of 2 to 3 mm., and shows, on its surface, branching folds (plicae palmatae, arbor vitae). The surface is lined by a tall columnar epithelium; in fixed material the oval nuclei are at the base of the cells (Fig. 499) with mucus in their apical parts. In the mucosa numerous large glands are present which differ from those of the corpus in that they are extensively branched, and are lined with a mucus-secreting, tall, columnar epithelium. Some of its cells especially on the surface are ciliated. The canal of the cervix is usually filled with mucus. Very often some of the cervical glands are transformed into cysts which may reach the size of a pea—the so-called *nabothian follicles*.

The mucosa of the cervix does not take part in the menstrual changes. An increase in vaginal mucus (which comes largely from the cervix) has been described at about the middle of the cycle (Papanicolau). In pregnancy the cervical glands enlarge, proliferate, and accumulate large quantities of mucus. The connective tissue between them is reduced to thin partitions.

The outer surface of the portio vaginalis is smooth, covered with a mucous membrane similar to that of the vagina and consists of a stratified squamous epithelium with glycogen in its cells and a lamina propria with small papillae well separated from one another.

The transition between the columnar mucous epithelium of the cervical canal and the stratified squamous epithelium of the portio vaginalis is abrupt; as a rule the borderline is just inside of the external opening of the cervix. In some cases patches of columnar epithelium may extend for short distances upon the outer surface of the portio vaginalis, forming so-called "physiologic erosions"; in others the vaginal end of the cervical canal has stratified epithelium.

Endometrium in Early Pregnancy. Great advances in our knowledge of early stages of placentation in man have been made by Hertig and Rock (1941, 1945) and in primates by Streeter and his colleagues, Wislocki, Hartman and Heuser, at the Carnegie Institution. Their papers as well as the extensive monograph on the human and monkey placenta by Wislocki and Bennett should be consulted for the details and peculiarities of human placentation.

The changes which occur in the first week after fertilization of the ovum have not been observed in man and can only

Fig. 499. Epithelium of the endocervix of a woman of forty-six years, fourteen days after the beginning of the last menstrual period. 510 ×. After Stieve.

Fig 500. Sagittal section through the posterior half of the portio vaginalis uteri and the fornix vaginae of a young woman: *F*, Fornix; *M*, muscle coat of the cervix; *M'*, muscle coat of the vagina; *O*, external orifice of the uterus; *S*, mucosa of the cervix; *S'*, mucosa of the vagina; *d*, *d'*, cervical glands; *c*, cylindrical epithelium; *f*, lymph follicle; *pc*, beginning of the stratified squamous epithelium with papillae; *v*, veins. 10 ×. After v. Ebner, from Schaffer.

be inferred from observations on monkey ova. After fertilization, segmentation occurs as the ovum moves down the tube and enters the uterus. Unlike the monkey ovum, however, the human ovum burrows into the endometrium, presumably through the activity of the outer layer of cells of the blactocyst. These cells will also

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help to form the *placenta*, the organ for the transfer of nutritive materials from the maternal circulation to the embryo and the removal of its waste products. These nourishing cells are therefore called the *trophoblast*.

At the time the ovum enters it, the endometrium may resemble that in Fig. 491 *b*, the glands are distended with glycogen and a mucoid, there are lipoid granules in epithelium and stroma and the latter is edematous.

The youngest human ovum studied is believed to be about seven and one-half days old (Hertig and Rock, 1945). It shows the blastocyst attached to the endometrium and invading its stroma (Fig. 501). The advancing edge of the blastocyst consists of a multinucleated protoplasmic mass, the *syncytial trophoblast*. Closer to the primordium of the embryo proper the trophoblast is made up of distinct cells, the *cytotrophoblast*. In the next older specimen (nine days) found by these authors, the ovum has burrowed deeper into the endometrium and is almost covered with uterine surface epithelium. The syncytial trophoblast has increased in amount and intercommunicating lacunae have developed in it. Some of these contain blood liberated by the penetration of the trophoblast into the maternal vessels. This is the beginning of the utero-placental circulation on which the continued growth of the embryo depends. In all probability the cytotrophoblast is giving rise to syncytial trophoblast externally and has begun to form mesoblastic cells on its inner surface. The embryo proper at this time consists of a well defined epithelial disc; an amniotic cavity is beginning to develop.

In the eleven-day specimen of Hertig and Rock (Figs. 502, 503) the trophoblast has increased in amount and with the primitive mesoblast forms the *chorion*. There are more frequent communications between the lacunae in the syncytial trophoblast and the maternal vessels.

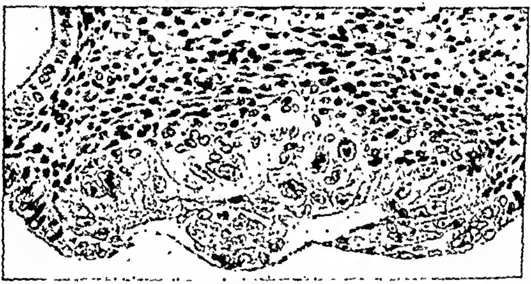

Fig. 501. Section of a seven and one-half day human ovum which is just embedding. Note broad layer of trophoblast penetrating endometrium. The faint cleft is probably the future amnion. 300 ×. Hertig-Rock. Courtesy of Carnegie Institution of Washington.

This invasion of the maternal blood vascular system by trophoblast becomes progressively extensive. The syncytium continues to enlarge the implantation cavity during the earliest weeks of pregnancy. Until term it is concerned with the absorption of nutriment and the excretion of wastes for the embryo and its membranes.

During the next few days the ovum enlarges by the growth of the trophoblast and the accumulation of fluid in the primi-

tive chorionic mesoblast. By the end of the second week the conditions shown in Figure 498 are reached, where a recon-

Fig. 502. A photomicrograph of a section through the implantation site of the eleven-day Hertig-Rock ovum. The entire thickness of the endometrium is shown. The glands are progravid, the stroma edematous and the superficial veins are dilated. Magnified 22 diameters. (Hertig and Rock, Carnegie Contrib. to Embryol., v. 29, 1941, Fig. 13.)

struction of the ovum described by Brewer (1937) is shown. The wide lacunae in the plasmodial trophoblast are in open communication with dilated maternal veins and there has been considerable extravasation of blood about the ovum. A cap of blood overlies the implantation site. Adjacent to the nutrient-laden maternal tissue, the trophoblast has grown more

Fig. 503. A photomicrograph from the same section as Fig. 502, magnified 160 diameters. The bulk of the ovum consists of irregular masses of trophoblast (syncytium) which is invading the endometrium. Within the syncytial trophoblast is the cellular trophoblast with obvious cell boundaries. The cells are arranged as a simple epithelium except for the clump at C. The cellular trophoblast immediately surrounds the primitive chorionic mesoblast in which the embryo is suspended. After Hertig and Rock, 1941, Fig. 14.

luxuriantly. The embryo it attached to the chorion on this side by a mass of mesenchymal cells, the future umbilical cord.

At several points on the chorionic wall, mesoblast is beginning to spread out into the trophoblast to produce the first *villi*; these outfoldings of the chorion serve to increase its absorbing surface. Fetal blood

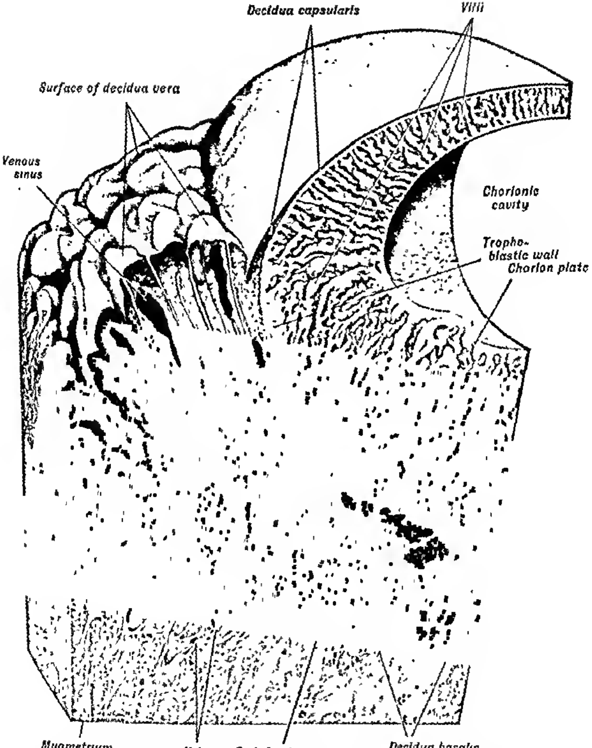

FIG. 504. The margin of the implantation site from a four weeks' pregnancy (Human Embryol Coll., Univ. of Chicago). The ovum is enclosed in the maternal decidua. The villi adjacent to the decidua basalis are long, have many secondary and a few tertiary branches, and are anchored to the decidua by a wall of cytotrophoblast. The decidua vera exhibits 3 zones; (1) a superficial compact zone with decidual cells; (2) a spongy zone of dilated and sacculated glands; (3) a basal zone of narrow glands which may be entirely absent. In the implantation site the compact zone has been obliterated by the developing ovum except for the attenuated decidua capsularis. The embryo and the amnion surrounding it are not shown. Courtesy of G. W. Bartelmez. Drawn by Miss Esther Bohlman. 17 ×.

vessels develop in the connective tissue core, which is covered by cellular and syncytial trophoblast (Fig. 506). The deeply staining syncytium has granular mitochondria and usually droplets of lipoid. In the cellular trophoblast, usually called Langhans cells in the villi, the mitochondria are elongate and sparse. The cytoplasm of these cells usually stains feebly and is rich in a labile form of glycogen.

During the third week the cellular trophoblast at the tips of the villi begins to grow rapidly and, mushrooming out, serves to anchor the villus to the decidua basalis. As this process continues the cellular proliferations of adjacent villi merge and thus an outer wall of trophoblast is formed through which the maternal vessels communicate with the space between the villi. This *intervillous space*, which *has developed from the trophoblastic lacunae* and the dilated maternal veins, is bounded throughout pregnancy by normal or degenerated trophoblast except where it communicates with maternal vessels. It contains more or less maternal blood; the villi absorb nutriment from it and excrete wastes into it. The development of a functional vascular system during the fourth week makes possible the nutrition of the rapidly growing embryo.

Figure 501 shows the relations of chorion and endometrium at the end of the fourth week. The implantation site occupies only a small part of the endometrium, all of which except for a basal zone is sloughed off after delivery. During pregnancy it has accordingly been called the *decidua* and it is divided into three regions: overlying the ovum is the *decidua capsularis;* underlying it is the *decidua basalis* which comprises the maternal component of the placenta; the rest of the endometrium as far as the internal os uteri is the *decidua vera.*

The vera continues to thicken during the first eight or ten weeks of pregnancy as the glands become more dilated with secretion and edema fluid accumulates. The stroma cells in the superficial one-third round up and enlarge, often becoming epithelioid. The superficial zone so formed has narrow glands and is called the *stratum compactum* in contrast to the underlying *stratum spongiosum* which may extend to the myometrium, or, as in Figure 504, there may be an intervening *stratum basale* of narrow tortuous glands and dense stroma.

The villi of the inner wall grow and branch profusely; together with the *chorionic plate* from which they arise, they constitute the *chorion frondosum.* The superficial villi, on the other hand, soon appear stunted and during the third month they degenerate, leaving the chorionic plate smooth; this is the *chorion laeve.*

The amnion closely enswathes the embryo for the first two months and there is a large cavity between amnion and chorion, the *exocoelom.* Early in the third month the amniotic fluid increases rapidly and the amnion is soon in contact with the chorion on all sides. As the membranes fuse the exocoelom is obliterated (Fig. 505, *a*). The uterus begins to enlarge and chorion laeve as well as all three deciduae are stretched and become thinner. But the placental villi are growing (Fig. 505) and the margin of the placenta becomes well defined. A section of the placenta from this stage shows, beginning at the fetal surface, first the amniotic epithelium, then the fused connective tissue of amnion and chorionic plate, followed by the villi cut in various planes. Some villi are fused to the decidua basalis and there are bands of necrotic trophoblast adjoining the decidua basalis. Here there are dilated and stretched glands and greatly modified coiled arteries which carry maternal blood to the intervillous space.

During the second half of pregnancy the terminal branches of the villi grow smaller and more numerous, but the rapid growth of the fetus involves an actual de-

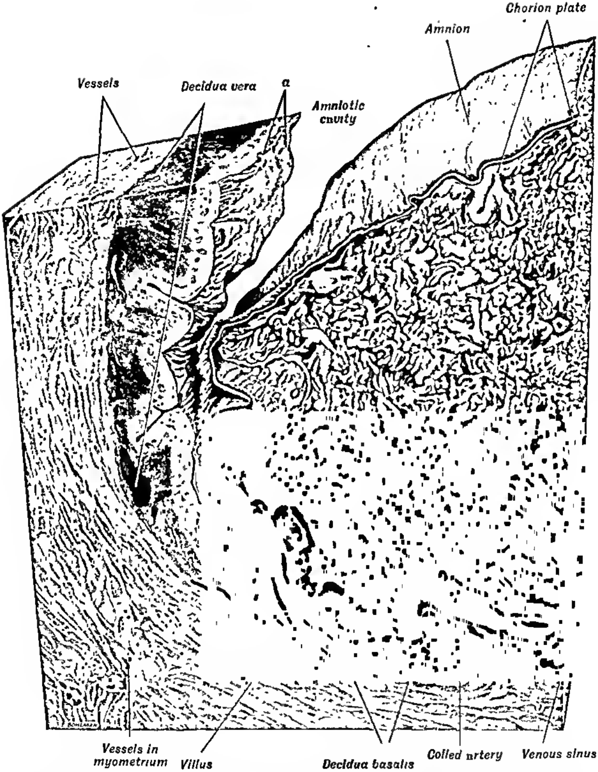

Fig. 505. Margin of the placenta from a sixteen weeks' pregnancy (Human Embryol. Coll. Univ. of Chicago). The growth of embryo and amnion as well as the enlargement of the uterus as a whole have changed the relations shown in Fig. 501. The connective tissue of amnion and chorion have fused everywhere, obliterating the exocoelom. Decidua capsularis and chorion laeve have fused with the stretched and regressing decidua vera, obliterating the uterine cavity as far as the internal os. The placental villi have grown and branched profusely, increasing the size of the placenta The end of each villous trunk remains attached to the decidua basalis and the small twigs float freely in the fluid of the intervillous space. The placenta now has a well-defined margin where the villi are shorter and there are wide communications between intervillous space and maternal veins. *a*, Fused amnion and chorion. Courtesy of G. W. Bartelmez. Drawn by Miss Esther Bohlman. 7.5 ×.

crease in the thickness of the placenta with an increase in its circumference. By the fifth month most of the cellular trophoblast (Langhans cells) has differentiated into syncytium. The syncytium thins out and in many spots overlying fetal capillaries it becomes a delicate membrane.

The detailed cytology and histochemistry of the trophoblast at various stages of pregnancy are given by Wislocki and Bennett who suggest that the steroid hormones of the placenta are produced by the syncytial trophoblast and the gonadotrophic hormone by the cellular trophoblast. (See also Dempsey and Wislocki.)

Textbooks of embryology and obstetrics must be consulted for more details on the development of the placenta and its circulation.

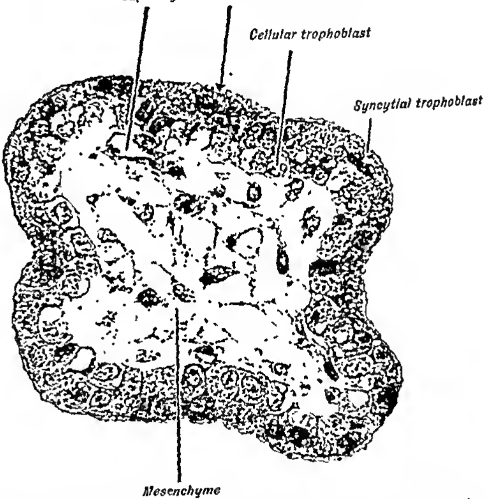

Fig. 506. Section of a villus from a three and a half weeks' pregnancy. The syncytium with its fuzzy brush border forms the outermost layer; within it is a continuous layer of cellular trophoblast which in turn surrounds the loose mesenchyme with its capillaries. Courtesy of G. W. Bartelmez. Photomicrograph 590 ×.

Vessels and Nerves. The larger branches of the uterine artery run chiefly in the stratum vasculare of the myometrium. From here radial branches run directly to the mucosa and form "coiled arteries." During progravid stages these vessels grow and become progressively more tortuous, soon reaching the surface and forming a rich capillary net. The basal zone is supplied by arterioles from the adjacent muscle. In the myometrium the capillaries have a peculiarly thick endothelium and sometimes a very small lumen. The veins form plexuses in the deeper layer of the lamina propria mucosae. Another plexus of very large veins without valves is found in the stratum vasculare of the myometrium.

Pregnancy causes irreversible changes in the

vessels. In a uterus which has been pregnant, the wall of the vessels at the placental site shows an irregular thickening of the intima with neoformation of smooth muscle, while in the media the muscle is largely substituted by coarse elastic networks.

The lymph vessels of the myometrium are readily demonstrated but their presence in the endometrium has been questioned.

The nerves of the uterus are for the most part nonmyelinated, although many fine and some thick, myelinated fibers occur also. They form the plexus uterovaginalis and are connected with a sympathetic "cervical" ganglion, located in the lateral, upper wall of the vagina. The ganglion contains, besides nerve cells, a considerable quantity of chromaffin elements. Inside the myometrium no nerve cells are found. Branching bundles of nerve fibers accompany the blood vessels and supply the muscular elements of the latter and of the myometrium. Some branches run through the mucosa toward the epithelium. The endings of the uterine nerves are not known exactly, but there is good physiologic evidence for afferent and efferent fibers to the myometrium. There is, however, no intrinsic coordinating mechanism like that of the gut. The rich innervation suggests an interplay of nervous and hormonal effects normally, even though in transplants the characteristic myometrial and vasomotor responses can be obtained under conditions that appear to rule out the nervous system completely.

VAGINA

The vagina arises from the distal ends of the müllerian ducts, fused in the midline; the outer part develops from the urogenital sinus. Its lower end is marked by a dorsal, transverse, semicircular fold of the wall, the hymen. The wall of the vagina consists of three layers: (1) the mucous membrane; (2) the muscular coat, and (3) the adventitial connective tissue.

The adventitial coat is a thin layer of dense, connective tissue which merges into the loose connective tissue joining the vagina to the surrounding parts. In this connective tissue there are a large venous plexus, nerve bundles, and small groups of nerve cells.

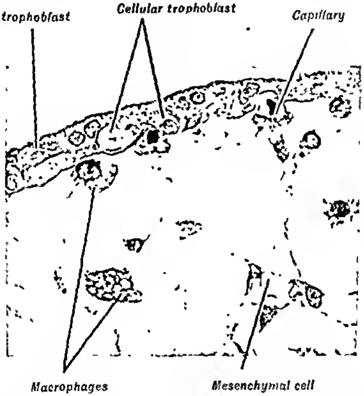

Fig. 507. Section of a villus from a four months' placenta. The syncytium is practically the same as in Fig. 506, but most cells of the cellular trophoblast have become syncytium. Free macrophages (Hofbauer cells) are common in the mesenchyme. Capillaries are closely applied to the syncytium. Courtesy of G. W. Bartelmez. Photomicrograph 590 ×.

The interlacing smooth muscle bundles of the middle layer are arranged circularly and longitudinally; the *longitudinal* bundles are far more numerous, especially in the outer half of the layer. The interstitial connective tissue contains *abundant*, coarse, elastic networks. Striated muscle fibers form a ring-shaped sphincter around the introitus of the vagina.

The mucous membrane consists of a lamina propria and an epithelium on the layer and become condensed in the walls of the blood vessels. Accumulations of *lymphocytes* are numerous and sometimes lymph nodules are found. Lymphocytes are always seen migrating into the epithelium. The deeper layers of the lamina propria contain dense plexuses of small veins.

The epithelium is of the stratified squamous variety and has a thickness of 150 to 200 μ. Under normal conditions the

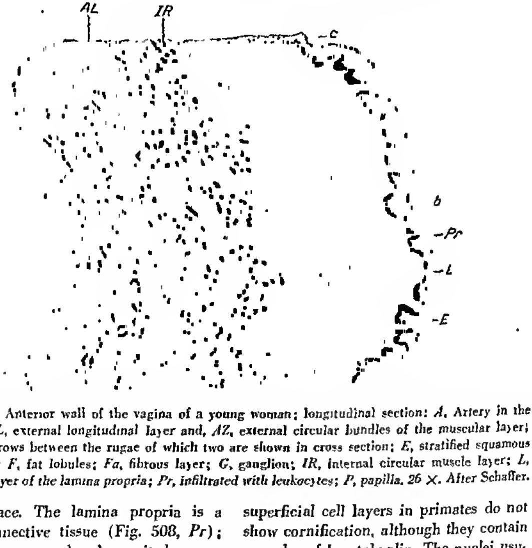

Fig. 508. Anterior wall of the vagina of a young woman; longitudinal section: *A*, Artery in the muscle; *AL*, external longitudinal layer and, *AZ*, external circular bundles of the muscular layer; *a*, *b*, *c*, furrows between the rugae of which two are shown in cross section; *E*, stratified squamous epithelium; *F*, fat lobules; *Fa*, fibrous layer; *G*, ganglion; *IR*, internal circular muscle layer; *L*, papillary layer of the lamina propria; *Pr*, infiltrated with leukocytes; *P*, papilla. 26 ×. After Schaffer.

free surface. The lamina propria is a dense connective tissue (Fig. 508, *Pr*); toward the muscular layer it becomes looser and this part may be considered as a submucosa. While in the anterior wall of the vagina papillae are scarce and small, in the posterior wall the lamina propria sends numerous high papillae deep into the covering epithelium. Immediately under the epithelium there is a dense network of fine elastic fibers; from here fine fibers run downward to the muscular superficial cell layers in primates do not show cornification, although they contain granules of keratohyalin. The nuclei usually remain stainable and the cells become loaded with glycogen and fat. In a prolapsed vagina, when the mucous membrane is exposed to air, the superficial cells are cornified as in the epidermis.

Glands are missing in the vagina; exceptionally, some few glands of the cervical type are found in the fornix. The mucus, *lubricating the vagina, originates*

from the cervix and is made acid by the fermentative action of bacteria on the glycogen from the vaginal epithelium.

The *hymen* is a fold of the mucous membrane with a thin connective tissue core and stratified squamous epithelium on both surfaces.

In spayed animals the vaginal epithelium is very low, whereas epithelial proliferation is clearly associated with a high level of the ovarian hormone, estrin. The estrin response of the vaginal epithelium in the spayed rat and mouse remains the best quantitative test for the hormone.

In primates, partly because of the normal presence of bacteria, the vaginal contents vary so greatly as to be of little value in diagnosing ovarian conditions. Sometimes a reduction in the number of leukocytes can be recognized at about the middle of the menstrual cycle which corresponds to the preovulatory phase in rodents, etc. A high estrin level is indicated by the thick epithelium and occasionally by erythrocytes of uterine origin. Usually three zones can be recognized. *A*, A basal zone of columnar and polyhedral cells with many mitoses; *B*, a narrow and variable zone with flattened cells sometimes containing granules that resemble keratohyalin; and *C*, an outer zone of squamous cells loaded with glycogen and fat. The nuclei of these cells stain readily in most cases. During menstruation the microscopic picture of the vaginal lavage is dominated by erythrocytes, normal and hemolyzed, and leukocytes. Blood soaked fragments of endometrium may occur in normal women, but blood clots are abnormal.

In sexually inactive states like childhood and old age, a vaginal proliferation is readily produced by the administration of estrin. This has proved to be a valuable method of clearing up vaginal infections.

In *gestation* the smooth muscle fibers enlarge and recede from one another. The connective tissue elements—the collagenous bundles and the cells—enlarge and are arranged more loosely. The blood vessels, especially the veins, also enlarge and the capillary endothelium swells. The thickness of the epithelium increases considerably during the first half and then diminishes greatly.

In various mammals (rat, guinea pig, opossum, etc.) the cyclic changes in the vaginal epithelium can be correlated with the ovarian and uterine changes, so that it is simply necessary to examine washings from the vagina to make a diagnosis of the stage in the ovarian cycle. The cyclic vaginal changes in the rat and guinea pig are as follows: During the rapid growth period of the graafian follicle the epithelium grows rapidly, so that 10 to 15 layers can be recognized, and leukocytes which were previously abundant disappear from the mucous membrane. A thick layer of squamous cells forms, in which the nuclei can no longer be stained. At about the time of ovulation the squamous cell layer is sloughed and leukocytes swarm through the epithelium in vast numbers. The cheesy mass which fills the lumen is discharged In the guinea pig the vaginal orifice is sealed by an epithelial proliferation. During the rest of the cycle the epithelium is of the low stratified squamous type and leukocytes continue to pass through it.

Vaginal lavages can accordingly be assigned to definite stages of the estrus cycle.

I—*Proestrus* associated with rapidly growing follicles. Leukocytes disappear from the lavage and only nucleated epithelial cells are present.

II—*Estrus* associated with ripe follicles. All nucleated epithelial cells are gradually replaced by squamous "plates" with no visible nuclei. The female will mate only during this stage, rarely during Stage I

III—*Metaestrus* The vagina is filled with a mass of sloughed epithelial plates, leukocytes begin to appear and rapidly become numerous. Ovulation occurs during this stage or between Stages II and III.

IV—*Diestrus* Until the beginning of the next cycle the lavage has nucleated epithelial cells, squamous plates and large numbers of leukocytes.

THE EXTERNAL GENITALIA

The outer sexual organs comprise the clitoris, the labia majora and minora, and certain glands which open into the vestibulum vaginae.

The *clitoris* corresponds embroyologically to the dorsal part of the penis; it consists of two small, erectile, cavernous bodies, ending in a rudimentary glans clitoridis, which is covered by the mucous membrane of the vestibulum vaginae, the space flanked by the labia minora. Into this space the vagina and the urethra open; it is lined with stratified squamous epithelium. Around the opening of the urethra and on the clitoris several small glands are arranged—*glandulae vestibulares minores;* they resemble the glands

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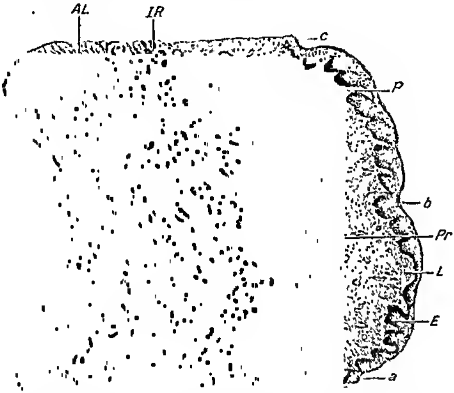

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The outer genital organs are richly supplied with sensory nerve endings. The epithelium contains the usual, free nerve endings. In the papillae, Meissner corpuscles, in the subpapillary layer, genital corpuscles are scattered. In the deeper parts of the connective tissue of the labia majora and in the cavernous bodies of the clitoris, pacinian corpuscles have been found.

CORRELATIONS IN THE FEMALE REPRODUCTIVE SYSTEM

There is some evidence that hormones are concerned in the embryonic development of the reproductive tract. The changes of puberty are under hormonal control and estrogens are probably the *immediate agents* concerned. During the period of sexual activity the ovarian hormones, *estrone* (*estrin*) and *progesterone* (*progestin*), control the tubal, uterine, and vaginal changes.

The ovary has intimate reciprocal relations with the pars distalis of the hypophysis. The *follicle stimulating* and *luteinizing* hormones together cause the preovulatory growth and rupture of ovarian follicles; the increased secretion of these hormones is caused by the action of estrogen and possibly progesterone on the pituitary. (See discussion by Hisaw.) Other endocrines are also involved in the control of pregnant and nonpregnant cycles. Psychic states may influence the menstrual rhythm in women and direct relations between the central nervous system and the hypophysis have been demonstrated. The complex interrelationships which control the reproductive organs are reviewed in detail in "Sex and Internal Secretions," edited by E. Allen.

In the vast majority of mammals, mating occurs only when the female is in a characteristic receptive condition ("in heat" or "estrus"). In primates there is no definite period of heat. In the human species the most obvious external indications of the reproductive cycle are hemorrhages. The menstrual cycle is, however, fundamentally similar to the estrus cycle, if it is divided into pre- and postovulation phases. Under optimal conditions the correlated ovarian and uterine changes are

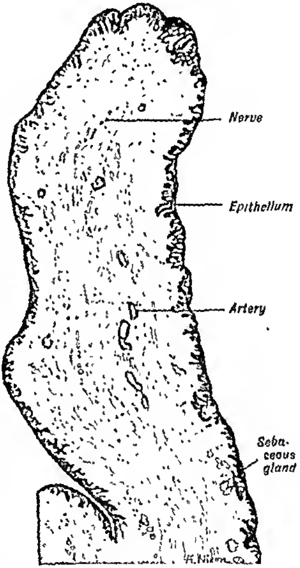

Fig. 510. Cross section of labium minus of a woman of thirty-four years. 10 ×.

more or less as indicated in Figure 497, which illustrates nonpregnant cycles. Thus ovulation may occur from seven to twenty-two days after the onset of a menstrual flow and the endometrium will vary accordingly. One cycle may be twenty-four days in length, the next twenty-eight or thirty-two days.

The changes of the follicular phase are definitely associated with estrin which

of Littré found in the male urethra, and contain mucous cells.

Two larger glands, the *glandulae vestibulares majores*, or *glands of Bartholin*, each the size of a bean, are located in the lateral walls of the vestibule. They open on the inner surface of the labia minora. They are of tubulo-alveolar character, closely correspond structurally to the bulbo-urethral glands of men, and secrete a similar lubricating mucus. After the thirtieth year they gradually begin to undergo involution.

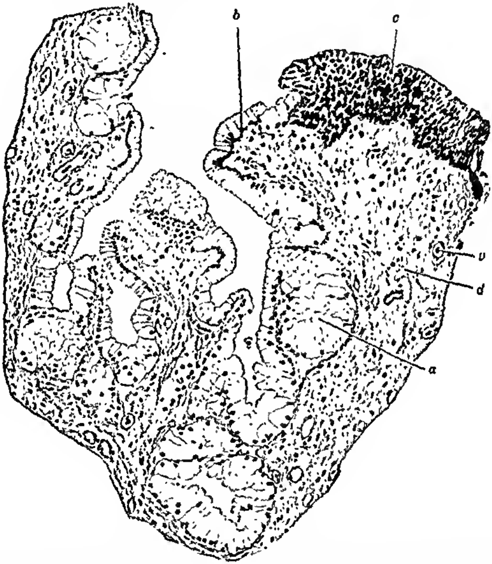

Fig. 509. Gland of Bartholin. A large duct with patches of stratified columnar epithelium (*c*) gives off smaller branches lined with columnar mucous cells (*b*) and continuing into tubulo-alveolar terminal portions; these are lined with large mucous cells (*a*); *d*, interstitial connective tissue; *v*, blood vessel. 281 ×, reduced to ⅔. (A.A.M.)

The *labia minora* are covered with stratified squamous epithelium and have a core of spongy connective tissue with fine elastic networks and with a large quantity of blood vessels, but without fat cells. It forms high papillae penetrating deep into the epithelium; the latter contains pigment in its deeper layer, while on the surface a thin horny layer is present. Numerous, large, sebaceous glands are found on both surfaces (Fig. 510); they are devoid of hair.

The *labia majora* are folds of skin with a large amount of fat tissue and a thin layer of smooth muscle, as in the scrotum. The outer surface is covered with hair, while the inner is smooth and hairless. On both surfaces sebaceous and sweat glands are numerous.

After that they both grow without dividing and are transformed into primary spermatocytes (*Fig.* 483, *b*) and primary ovocytes (Fig. 483, *b'*) respectively—period of growth. Finally, both spermatogenic cells and ova pass through the period of maturation; this is characterized by two peculiar "meiotic" or "maturation" divisions, which rapidly succeed each other. In both cases the ultimate result is a sex cell ready for fertilization, with a nucleus containing but one half of the somatic number of chromosomes.

But the spermia are produced anew continuously, or at certain intervals, during the whole sexual life of the individual, while the ova in primates probably complete their period of multiplication shortly before birth or in the first weeks of extrauterine life. Then, very slowly, one by one, they undergo the maturation divisions. The vast majority of them degenerate before they reach this stage.

It has been shown in various rodents, that new eggs are produced from the germinal epithelium throughout infancy and even during sexual maturity.

The embryonic origin of the Sertoli cells in the testis and of the follicular epithelium in the ovary is the same. Their functional rôle in the sexual gland of the adult is probably also similar. Both protect the developing sex cells and furnish them suitable nutritive material. The possibility of production of spermatogenic cells by Sertoli cells has been mentioned above; a similar production of new ova by the follicular cells in the mammals has not been observed.

The interstitial cells are far less constant in the mammalian ovary than in the testis. They probably arise from the theca interna of atretic follicles; in the human ovary they may even be totally absent. The morphologic relations between the two sex glands and their similarity are best revealed by the study of their embryonic histogenesis.

Histogenesis of the Sex Glands. The primordia of the gonads arise as strips of thickened, mesodermal epithelium on the surface of the urogenital folds—the *genital ridges.*

The epithelium of the genital ridge, the *germinal epithelium*, at first has the same structure

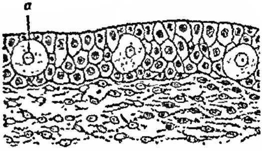

Fig. 511. Diagram of the primordium of the gonad in the early indifferent stage, in the form of a "germinal plate": *a*, Primordial sex cell. Redrawn after A. Kohn.

in both sexes. This is the so-called "indifferent" stage of the gonad (*Fig.* 511), although the sex of the embryo is probably determined at the time of fertilization.

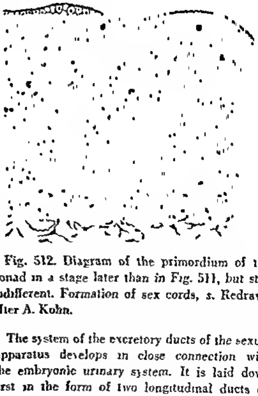

Fig. 512. Diagram of the primordium of the gonad in a stage later than in Fig. 511, but still indifferent. Formation of sex cords, *s*. Redrawn after A. Kohn.

The system of the excretory ducts of the sexual apparatus develops in close connection with the embryonic urinary system. It is laid down first in the form of two longitudinal ducts on both sides—the *duct of Wolff* and the *duct of Muller;* they arise from the mesoderm which lines the celom Both open into the urogenital sinus.

When the thickened germinal epithelium in the male embryo develops into the seminiferous tubules, the wolffian duct comes into connection with them and furnishes the excretory system

is abundant in young human corpora lutea. The interval between the follicular phase and the lutein phase involves a superficial endometrial hyperemia and sometimes diapedesis of erythrocytes with *the appearance of blood in the vagina. If* the follicular phase has been produced with estrin in a spayed female, progravid changes will appear on the administration of progestin. It would seem that the ischemic phase with the associated involution of the endometrium is due to a reduction in the available ovarian hormones and there is evidence that the corpus luteum is regressing at this time. It has been suggested that the ovarian hormones, having been kept at a definite high threshold for a definite time, inhibit the gonadotrophic activity of the distal hypophysis and this is the reason for the diminished production by the ovary (Corner). Whether the hemorrhagic changes involve other hormonal mechanisms or not remains to be seen.

The estrus state appears at intervals which vary according to the species (four to five days in the rat, fifteen to seventeen days in the guinea pig, about three weeks in the sow, twice yearly in the bitch and ewe). Wild species like the opossum and ground squirrel, which have a definite breeding season, exhibit several cycles if pregnancy is prevented. In the absence of pregnancy, the cycles in domestic animals continue throughout the year when food and temperature conditions are kept optimal.

During estrus graafian follicles are approaching maturity, the content of estrin in the blood is high and its action is manifested by the behavior of the animal and the anatomical and physiological reactions of the reproductive tract. The succession of events in the rat is as follows (Long and Evans, 1922). As the follicles approach maturity the motility of tubes and uterus increases and there is a rapid secretion of fluid by the uterine glands, so that the organ is greatly distended. *These conditions are important for the transport of spermatozoa* (Rossman) and the active proliferation of vaginal epithelium is interpreted by Papanicolaou as an adaptation for mating. The changes of these phases of the cycle (proestrus and estrus) have been produced experimentally by estrogens in spayed animals. In the normal animal ovulation is spontaneous. After this event the ova pass into the ampulla of the tube and reach the uterus on the fourth day. In the interim the uterus has collapsed and the vaginal epithelium has been sloughed. As the corpora lutea develop, the uterine muscle becomes less active and then the ova, *if fertilized*, are regularly spaced along the uterine horns. If mating has not occurred, regressive changes appear in the *corpora lutea, the uterus and the vagina*, and then a new cycle begins.

In the rabbit, ovulation is not spontaneous. An isolated adult female in heat has about ten large graafian follicles in the ovaries; the number characteristic for the species is apparently determined by the amount of gonadotrophic hormone available. After mating these follicles grow rapidly and in about ten hours ovulation occurs. The stimulus of coitus involves the portio vaginalis of the cervix and an erogenous area of skin on the flanks. The resulting nervous impulses pass to the spinal cord and brain. Through a center in the hypothalamus the pars distalis of the hypophysis is stimulated to secrete gonadotrophic hormones which activate the ovaries. If one and one quarter hours elapse between mating and hypophysectomy, enough gonadotrophic substance enters the blood stream to induce ovulation (Fee and Parks). Under these conditions corpora lutea develop, but they promptly regress (Smith and White) and, in the consequent absence of progesterone, the fertilized eggs die. Such corpora can be maintained in a functional state either by gonadotrophins or by estrin (Westman, 1937).

COMPARISON OF THE STRUCTURE OF THE TESTIS AND THE OVARY

The sex cells in the testis and ovary of the embryo arise from the same germinal epithelium. In the testis of the adult it persists as a flattened mesothelium on the outer surface of the albuginea. On the surface of the adult ovary it remains more or less unchanged.

The spermatogenic cells and the ova undergo comparable developmental transformations; both pass through a period of intense mitotic activity of the ordinary somatic type—period of multiplication.

beginning of puberty will have to be explained through neoformation from the small indifferent epithelial cells, that is, from the future cells of Sertoli.

does not commence at once. With each of several successive attempts the spermatogenic cells reach a higher level. Finally, functionally adequate spermia are produced and from now on

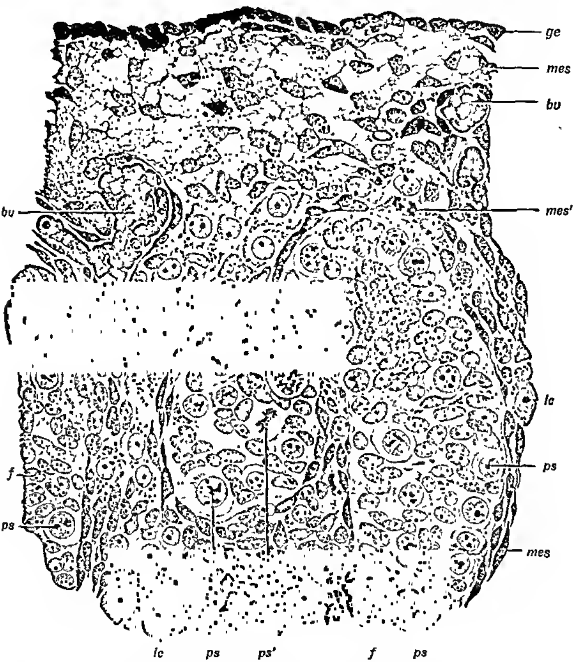

Fig. 514. Testis of human embryo of 70 mm. Seminiferous tubules without free lumen, with two kinds of cells: *ps* and *ps'*, Primordial sex (spermatogenic) cells and, *f*, follicular (future Sertoli) cells. Between the tubules abundant interstitial tissue, with mesenchymal cells (*mes*, *mes'*) and interstitial cells (*ic*); *bv*, blood vessels, *ge*, germinal epithelium. 780 ×, reduced to ¾. (A.A.M.)

At the onset of puberty, the round sex cells between the follicular epithelial cells begin to proliferate and increase in numbers. They have the character of spermatogonia as they appear in the adult. The indifferent "follicular" epithelial cells between them decrease relatively in numbers They gradually assume the structural character of the Sertoli cells. Spermatogenesis spermatogenesis continues without interruption throughout the whole period of sexual life.

The beginning of the sexual differentiation may be determined in female embryos at the stage of eight weeks. The solid, medullary, epithelial mass is loosened by mesenchyme, which grows into it and separates the epithelial strands into thin, irregularly arranged, *medullary cords*

of the male sexual gland, while the duct of Müller involutes, leaving only small rudiments. In the female the same mesodermal primordium of the gonad is transformed into the ovary. Here the wolffian ducts regress, whereas the mullerian ducts furnish the oviducts, the uterus, and the vagina.

The germinal epithelium of the "indifferent" gonad consists of two kinds of cells. The majority is represented by small, irregularly cuboidal or columnar elements arranged in a simple pseudostratified layer or in several layers. They contain threadlike mitochondria and show numerous

In the male human embryo the histologic differentiation typical for the testis becomes recognizable at about seven weeks. The outlines of the sex cords become more distinct, they are transformed into the convoluted seminiferous tubules and gradually develop a basement membrane at their surface. The seminiferous tubules anastomose by their peripheral (proximal) ends, while their deeper (distal) ends open through the rete testis into the tubules of the mesonephros (the future coni vasculosi) and beyond into the wolffian duct (the future vas deferens, Fig. 513).

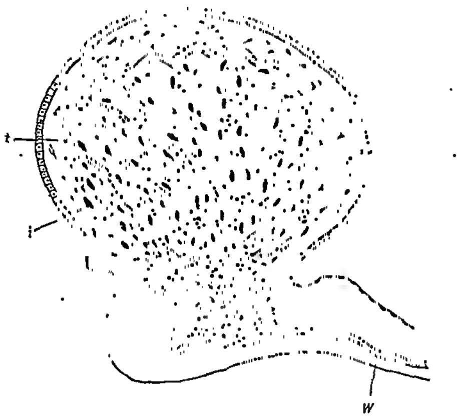

Fig. 513 Diagram of development of the testis: *t*, Seminiferous tubules; *i*, interstitial cells; *r*, rete testis; *d*, ductuli efferentes (urogenital junction); *W*, wolffian duct. Redrawn after A. Kohn.

mitoses Between them, large spherical elements are scattered—the *primitive sex cells* (Fig. 511, *a*); they are far less numerous and their total number can be counted in some species of animals. They have a clear transparent cytoplasm, a large vesicular nucleus and granular mitochondria. They also may show, occasionally, mitotic divisions.

There is a massive penetration of the subjacent mesenchyme by epithelium in which the sex cords develop. They consist of the same two cell types as the germinal epithelium. The deepest parts of the epithelial mass consist of smaller cells arranged in the form of an epithelial network which later receives a lumen—the rete. It is present in both sexes.

Throughout the whole prepubertal period, the seminiferous tubules seem to contain the same two apparently independent cell types. The small epithelial cells form the vast majority and are arranged radially; no distinct lumen is found in the axis of the tubules and the bodies of these elements seem to fuse here into a syncytial network. *The primary sex cells are scattered* singly between the small epithelial cells (Figs. 514, 515).

It has been claimed that the primary sex cells of the embryo all disappear and that at a certain stage the prepubertal seminiferous tubules contain but one kind of cell—the small epithelial elements. Should this be definitely confirmed the appearance of new spermatogenic cells at the

The modification of sex in chick embryos through the injection of sex hormones is described by Willier, Gallagher and Koch. Greene and Ivy report similar findings with rats (but see Moore's studies on opossums).

Interstitial Cells. The interstitial cells of the testis appear first in the human embryo of 19.5 mm. They arise through transformation of the mesenchymal cells, although some investigators claim that the cells of the sex cords may also be a source of interstitial cells.

In the second half of embryonic life, the interstitial cells in the testis are arranged in broad strands between the seminiferous tubules (Fig 513, *i*). Toward the end of gestation their quantity decreases relatively and remains small in the prepubertal period At the beginning of puberty they again become more conspicuous.

In the interstitial tissue of the human ovary, the first interstitial cells were described in embryos of 4 cm Their mesenchymal origin seems to be beyond doubt, although some recent investigators derive them from the germinal epithelium. After a period of numerical increase, they undergo involution or are transformed into common connective tissue cells In embryos of 7 cm. another period of progressive development begins in the medulla of the ovary; the hypertrophy of the mesenchymal cells is more pronounced. This period results again in involution shortly after birth. The third generation of interstitial cells appears in the cortex around the follicles before the second has disappeared. In postembryonic life there are no cells resembling interstitial cells except the theca cells of atretic follicles.

Origin of the Definitive Sex Cells. A disputed question is the origin of the definite sex cells.

In some lower invertebrates (Ascaris) the sex cells are separated from the rest of the individual in very early stages of ontogenesis. This continuity of the sex or germ cells throughout *innumerable generations is called the* "germ track."

Many investigators endeavored to establish the existence of a germ track in mammals. Peculiar cells have been found in relatively early stages of development in the mammals (guinea pig) which have the aspect of ova—the primitive sex cells. They were first found in the mass of undifferentiated cells at the posterior end of the primitive streak and in the entoderm of the posterior part of the intestine These primitive sex cells were seen to migrate through the mesenchyme to the root of the mesentery and to enter

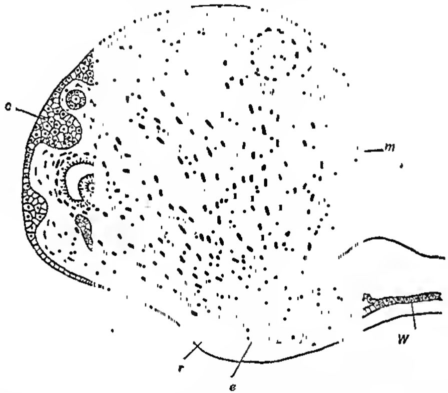

Fig. 516. Diagram of development of the ovary: *c*, Cortical substance with formation of follicles; *m*, medullary cords, homologues of the seminiferous tubules in the testis in Fig. 513, and, like the latter, derivatives of the sex cords in Fig. 512; *i*, interstitial cells; r, rete ovarii; e, epoophoron (urogenital junction); *W*, wolffian duct. Redrawn after Kohn.

(Fig. 516). These are homologues of the seminiferous tubules and may become connected with the tubules of the mesonephros. They contain primitive sex cells (*primitive ova*) and may even show a transient formation of rudimentary follicles. But they gradually involute and in the adult ovary no trace of them can be found as a rule.

In human embryos of four to five months a new, thick, cortical layer of epithelium is formed (Fig. 517). This is the second proliferation period which produces the primitive cortex of the ovary. Mesenchymal strands with fusiform cells and blood vessels grow toward the surface, penetrate the epithelial cortex, and subdivide it into cell clusters of irregular form. The ova are surrounded by the smaller epithelial cells—the primary follicles. The germinal epithelium continues to add new cell masses to the cortex.

The female sex cells in the cortex of the embryonic human ovary show the same nuclear transformations as are found in the spermatogenic cells of the seminiferous tubules during the whole period of sexual activity. This can be seen distinctly in the cortex of the ovary of a human embryo of four and a half months (Fig 517). In the resting condition the nuclei of the *ova* have a reticular structure with a few larger chromatin particles and a nucleolus Such cells may be considered as at the end of the period of multiplication and correspond to the spermatogonia in the testis; accordingly, they have been named *oogonia* The numerous, smaller, epithelial cells surrounding them are the follicular cells of the future follicles and stroma cells.

The older generations of oogonia show the first stages of further development of the period of growth. These cells from now on have to be designated as primary ovocytes—homologues of the primary spermatocytes. Their body enlarges and the nuclear chromatin is arranged in the form of thin threads interlacing in the nuclear space (the leptotene stage, Fig. 517, *l*). Later, the threads, often joining one another side by side, are coiled up at one pole of the nucleus (synaptene stage, Fig. 517, *syn*). The pachytene and diplotene stages develop later in the same way as in spermatogenesis. Simultaneously with all these changes, each ovocyte is gradually surrounded by a concentric layer of small, squamous, follicular cells. Then the young follicles are separated by connective tissue strands.

After the diplotene stage is reached by the nuclei of the ovocytes, their further transformation may be discontinued for a long time. The nucleus of such ovocytes in the follicles contains a loose, pale network and a large chromatin nucleolus while, near the nucleus, a crescentic accumulation of mitochondria develops. After birth these ovocytes in the primary follicles gradually undergo the transformations described early in this chapter.

In the ovary of a human embryo of about 180 mm., the mesenchyme reaches the surface layer of the *germinal epithelium* and gradually forms the albuginea of the ovary which separates the deeper tissue—the definitive cortex—from the epithelial sheet on the surface. After this, according to the dominant opinion, additional ova are not formed from the germinal epithelium.

Thus the medullary cords of the ovary are homologous to the seminiferous tubules of the testis. The cortex of the mature ovary is an additional formation which is not found in the testis.

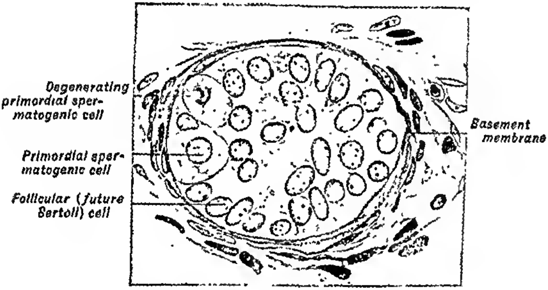

Fig. 515. Section of seminiferous tubule of testis of six months' infant. Iron-hematoxylin. 720 ×.

The modification of sex in chick embryos through the injection of sex hormones is described by Willier, Gallagher and Koch. Greene and Ivy report similar findings with rats (but see Moore's studies on opossums).

Interstitial Cells. The interstitial cells of the testis appear first in the human embryo of 19.5 mm. They arise through transformation of the mesenchymal cells, although some investigators claim that the cells of the sex cords may also be a source of interstitial cells.

In the second half of embryonic life, the interstitial cells in the testis are arranged in broad strands between the seminiferous tubules (Fig. 513, I). Toward the end of gestation their quantity decreases relatively and remains small in the prepubertal period At the beginning of puberty they again become more conspicuous.

In the interstitial tissue of the human ovary, the first interstitial cells were described in embryos of 4 cm Their mesenchymal origin seems to be beyond doubt, although some recent investigators derive them from the germinal epithelium. After a period of numerical increase, they undergo involution or are transformed into common connective tissue cells. In embryos of 7 cm. another period of progressive development begins in the medulla of the ovary; the hypertrophy of the mesenchymal cells is more pronounced. This period results again in involution shortly after birth. The third generation of interstitial cells appears in the cortex around the follicles before the second has disappeared. In postembryonic life there are no cells resembling interstitial cells except the theca cells of atretic follicles.

Origin of the Definitive Sex Cells. A disputed question is the origin of the definite sex cells.

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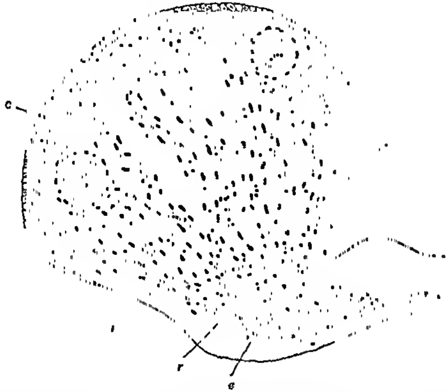

Fig. 516. Diagram of development of the ovary: *c*, Cortical substance with formation of follicles; *m*, medullary cords, homologues of the seminiferous tubules in the testis in Fig. 513, and, like the latter, derivatives of the sex cords in Fig 512; *i*, interstitial cells; *r*, rete ovarii; *e*, epoophoron (urogenital junction), *W'*, wolffian duct. Redrawn after Kohn.

the thickened celomic epithelium of the primordium of the gonad. In the description given above they were mentioned as one of the two cell types present in the germinal epithelium.

In many of the lower vertebrates (chick, reptiles, amphibians, fishes) cells with a similar history have been found. If, in young amphibian and chick embryos, the parts containing the primitive sex cells are excised on one side, the corresponding gonad does not develop or remains sterile. Although the primitive sex cells have not been traced back to the earliest stages of ontogenesis, the evidence seems to be in favor of the existence of a germ track in the other mammals and, perhaps, in man.

This conclusion is, however, far from being generally admitted. It has been pointed out by numerous investigators that the primitive sex cells sooner or later degenerate and disappear among the epithelial cells of the sex cords and follicles, especially in mammalian embryos. They claim that the definitive sex cells of the adult develop secondarily in the germinal epithelium, or, later, in the sex cords, through transformation of the common celomic epithelial cells. If this is true, then there is no germ track in the vertebrates. This conception of the nature of the definitive sex cells would gain strong support if certain observations mentioned above for the mature sexual glands could be confirmed—the neoformation of spermatogonia from Sertoli cells as described in the regeneration of the seminiferous epithelium after x-raying, the postpubertal neoformation of ova from the germinal epithelium

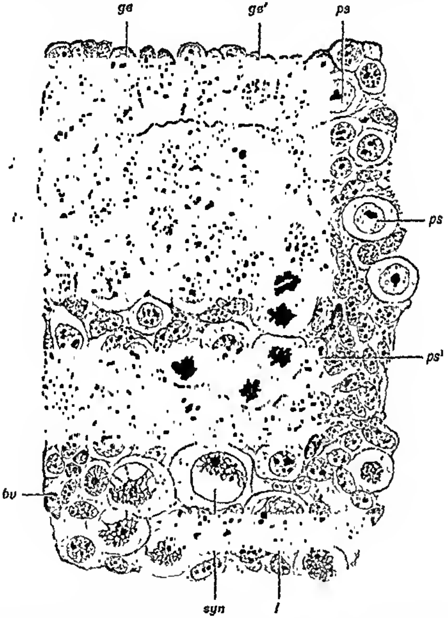

Fig. 517. Superficial layer of the ovary of a human fetus of 4.5 months: *ge*, Germinal epithelium with mitosis (*ge'*); *ps*, *ps'*, primordial sex cells (oval with mitosis; *l*, leptotene nuclei in growing ovocytes; syn, synaptene nuclei; *f*, follicular cells; *bv*, blood vessels. 780 ×, reduced to ⅘. (A.A.M.)

and the regeneration of the ovarian cortex after injuries.

It may be, however, that the apparently undifferentiated cells which give rise to definitive sex cells are primordial sex cells which have dedifferentiated during development and do not again differentiate until an adequate stimulus is given.

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Chapter XXVI

THE MAMMARY GLAND

The mammary gland undergoes extensive structural changes which depend on the age and sex of the individual and the functional condition of the sexual apparatus. In its structure and development, the mammary gland suggests somewhat a sweat gland. In man, only one pair develops normally and is laid down in the same manner in the embryos of both sexes. In males the gland involutes after birth. In females it continues to develop; it reaches its final development only at the end of pregnancy and remains in this condition until the end of the period of lactation, when it undergoes a partial involution; it becomes markedly atrophic after the menopause.

The Resting Mammary Gland. Each mammary gland of a woman consists of fifteen to twenty-five closely adjoining, irregular lobes radiating from the mammary papilla or nipple. These are separated from one another by layers of connective tissue and much adipose tissue. Each lobe is provided with an excretory duct 2 to 4.5 mm. in diameter which is lined by stratified squamous epithelium. This is the lactiferous duct which runs toward and opens on the nipple and has an irregular, angular form in cross section. Each duct under the areola—the pigmented circular area of skin surrounding the nipple—has a local dilatation, the *sinus lactiferus;* it then becomes constricted again and, curving toward the surface of the skin, opens at the summit of the nipple as an independent opening, 0.4 to 0.7 mm. in diameter.

Each lobe is subdivided by layers of connective tissue, rich in lobular masses of adipose tissue, into lobules of various orders. The smallest consist of elongated tubes or sacs, the alveolar ducts, which are covered by round evaginations, the alveoli (see following text).

The interlobular connective tissue is of the dense type. The intralobular connective tissue is much more cellular than the interlobular tissue and contains fewer collagenous fibers and practically no fat (Fig. 518, *B*). This layer of loose connective tissue about the ducts undoubtedly has a functional significance in providing an easily distensible medium for the hypertrophy of the epithelial portions of the organ during pregnancy and lactation.

The wall of the secretory portions, the alveolar ducts and the alveoli, consists of a basement membrane, a layer of myo-epithelial cells and, on the internal surface, a row of low columnar glandular cells. The myo-epithelial cells are especially prominent near the excretory ducts. These cells serve to associate the mammary gland morphogenetically with the sweat glands.

There has been much discussion as to the presence of *alveoli* in the nonlactating breast. According to most descriptions, the epithelial structures consist, during the resting phase, only of ducts and their branches. Some authors, however, believe that the resting breast always has a few alveoli which have budded off from the ducts and that these are grouped into small lobules. These pass over without definite boundaries into the primary ex-

cretory ducts by a simple constriction. The latter, in turn, gradually unite with similar ducts into larger and larger ducts which finally form the lactiferous duct; along its course the latter receives, directly, small ducts from the alveoli close to the nipple.

Each lobe of the *mammary gland* is thus an independent, compound, alveolar gland. The mammary gland is a conglomeration of a variable number of such independent glands—each with its own excretory or lactiferous duct which has its separate opening on the surface of the nipple (Fig. 519, *G*, *Gm*).

In a mature mammary gland periodical changes undoubtedly take place in connection with the sexual cycle of the ovaries and the uterus. The exact changes which take place have not been studied thoroughly in women. This is due to the difficulty in obtaining portions of the gland in healthy women and correlating their structure with the functional condition of the ovaries and uterus. To date the few studies which have been made are based mainly on postmortem material. This obviously does not help very much in elucidating the changes in normal women. It is probable that the changes in the breast which occur with menstruation consist in a hyperemia and perhaps edema of the interstitial connective tissue; the claim that the ductule and acinar epithelium undergoes a marked cyclic hyperplasia—on the order of the progravid changes in the endometrium—is probably unfounded. In laboratory animals, an extensive literature shows quite clearly that the cyclic changes in the breast of the female are intimately bound up with the functional state of the ovaries and uterus.

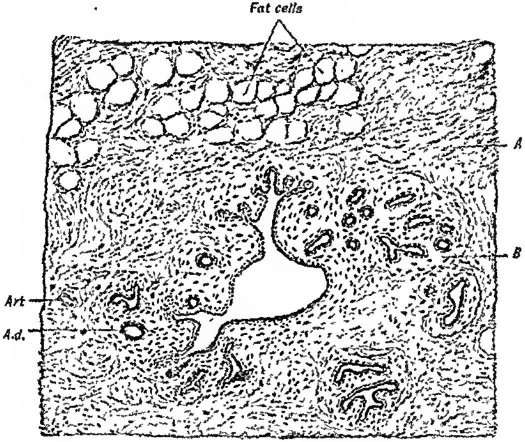

Fig. 518. Section through the mammary gland (resting state) of a thirty-seven-year-old woman. *A*, Interlobular and, *B*, intralobular connective tissue; *Art.*, artery; *A.d.*, alveolar duct. Hematoxylin-eosin-azure stain 75 ×.

The Nipple and Areola. The skin of the nipple and areola has tall, complex papillae. The epidermis is deeply pigmented, especially after the first pregnancy. Smooth muscles are located both circularly around the papilla mammae and along the length of the lactiferous ducts. In the papillary area are special accessory mammary glands; these are the

Fig. 519. Nipple of female breast in perpendicular section: *B*, Connective tissue stroma; *E*, epidermis; *G*, longitudinally cut mammary ducts, which open at the apex, *Gm*, and the sides of the nipple, *G'm*; *M*, cross section of circular smooth muscle bundles; *T*, sebaceous glands without hairs. 6 ×. After Schaffer.

Fig. 520. Hypertrophy of a lobule of the mammary gland from a woman in the fourth month of pregnancy. *D*, Duct; *A*, interlobular connective tissue; *B*, intralobular connective tissue. Hematoxylin-eosin-azure stain. 75 ×

areolar glands of Montgomery. They occupy an intermediate position between the true mammary gland and the sweat glands. Along the margin of the areola are large sweat and sebaceous glands which lack hairs or rudimentary hairs

(Fig. 519, *T*). These glands often open to the exterior by a common opening with the sweat glands.

The Mammary Gland During Gestation. From the time of implantation of the ovum in the uterus, progressive, deep-seated changes take place in the mammary gland. These develop during the period of gestation and can be separated into two phases. *During the first half of pregnancy* very rapid multiplication of the epithelium is noticed at the ends of excretory ducts, and secretory portions are formed which are devoid of a lumen, but are provided with pocket-like evaginations, the alveoli. The masses of interstitial fatty tissue disappear for the most part and make room for the hypertrophy of the epithelial structures (Fig. 520). Parallel to this process, an infiltration with round lymphoid cells begins in the surrounding interstitial tissue. During the

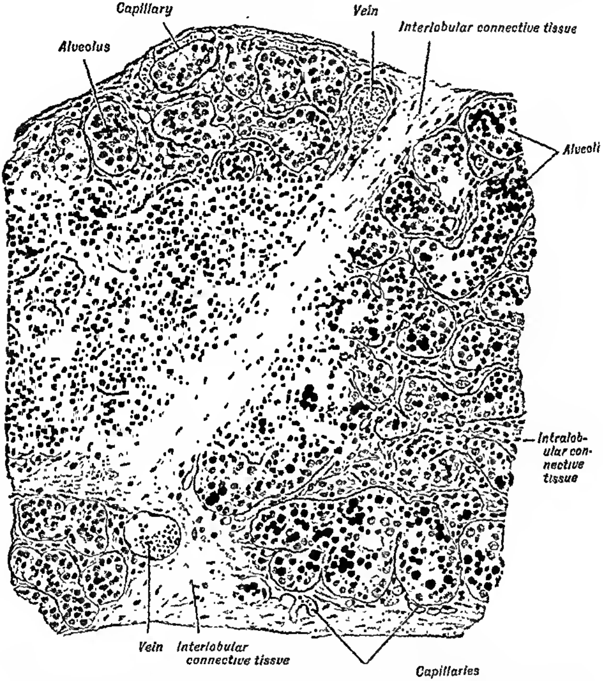

Fig. 521. Mammary gland of a woman in the sixth month of pregnancy, showing the beginning of secretory activity with osmic acid stained droplets of fat (black) in the hypertrophic epithelium. 187 ×. (A.A.M.)

second part of the pregnancy the multiplication of epithelial cells gradually decreases in intensity while the glandular cells gradually begin to produce a secretion (Fig. 521) and at the end of pregnancy the *colostrum* appears. During the first days after delivery the colostrum is replaced by milk and the infiltration of the stroma of the gland by lymphoid elements becomes less intense.

The Mammary Gland During Lactation. The different parts of the active mammary gland are usually not in the same functional state at the same time. and, therefore, their histologic appearance varies in different parts of the organ. In some places the secretory portions are filled with milk, their lumen is wide and the walls are dilated and thin; in others, on the contrary, the lumen is narrow and the epithelium forms a thick layer; in certain places the glandular elements may be greatly distended with secretion while in others they may appear more or less free of it.

The shape of the glandular cells fluctuates from cylindrical or conical to flat. The boundary between them is usually indistinct. If the cells are tall, then their distal ends, as in sweat glands, are often definitely separated from one another and project into the lumen of the alveoli as free, rounded, scalloped or star-shaped protrusions. The nucleus may be round or oval and is located at about the middle of the cell. If the cells are short, their free surface is usually more or less smooth; the nucleus here is frequently shrunken.

In the cytoplasm of the glandular cells are short or long rod-shaped or granular mitochondria; in the flattened cells they are few in number; in the tall conical ones they are more abundant (Fig. 522). At the base of the cells is some chromophil substance.

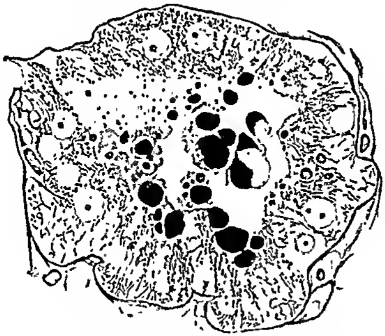

Fig. 522. Alveolus of a lactating mammary gland of a rabbit. The cells contain mitochondria and droplets of fat (stained black with osmic acid). The latter, with the adjacent protoplasm, are extruded into the lumen. 1000 ×. (A.A.M.)

The most characteristic expression of the secretory function is the drops of fat, sometimes of very considerable size, which accumulate mainly on the free surface of the cell body projecting into the lumen. After the dissolution of the fat in preparing sections, clear vacuoles remain in place of the fat drops. Sometimes, granules of albuminous substance, with which

fat had probably been mixed, also can be seen. Some authors have even described the presence of fat droplets in the nucleus, but this has not been confirmed sufficiently. It is unlikely that the mitochondria change into fat droplets. Cyclic changes in the Golgi net during the phases of secretion have been described.

Besides the fat drops in the peripheral portions of cells, round secretory granules and vacuoles of unknown, probably albuminous nature are sometimes seen. The fat drops, which accumulate in the end of the cell protruding into the lumen, pass out of the protoplasm; in preparations treated with osmic acid this can be observed directly and the free fat drops can also be noticed in the lumen of the alveoli (Figs. 521, 522). In preparations from which the fat has been dissolved, the resulting vacuoles appear in many places to be open to the outside, and the distal end of the cell, therefore, appears torn and uneven. The secretory granules of albuminous character have probably been dissolved previously.

It is possible, however, that the described process of secretion may, during very strong sucking, be accompanied by a partial disintegration of the glandular cell. The portion of the cell, filled with fat drops, which protrudes into the lumen of the alveolus sometimes becomes constricted off from the remaining, larger portion of the cell body which remains in its place. The detached part flows into the lumen where the remains of the protoplasm and albuminous granules dissolve and the drops become free. The glandular cell rapidly replaces the lost protoplasm and, after having again accumulated secretion, excretes it in the same way. This type of secretion is intermediate between the merocrine and the holocrine types and is called *apocrine*.

The epithelium of the excretory ducts is cubical or even low cylindrical in the small ones. Between it and the basement membrane, elongated, spindle-shaped myoepithelial cells are ordinarily seen. In the larger ducts the epithelium becomes taller and cylindrical; in the main lactiferous ducts it is stratified and is replaced by a stratified squamous epithelium at some distance from the opening on the nipple.

The interstitial connective tissue of the lactating mammary gland, which separates the lobes and lobules of various orders, is a rather dense connective tissue which is distributed in stripes of varying widths (Fig. 521). In the functioning gland the mass of the connective tissue is much less than that of the glandular elements. In addition to the collagenous fibers the interstitial substance contains elastic fibers which form dense networks, particularly along the external surfaces of the excretory ducts. Normally, there are no elastic fibers within the lobules.

In certain places, particularly in the peripheral portions of the organ near the nipple, the interstitial tissue is penetrated by peculiar shafts of smooth muscles which are connected with tendons of elastic networks.

Those portions of the interstitial connective tissue which extend between the small alveoli and directly cover the secretory portions have different properties—here the tissue is very rich in blood vessels, is much looser and contains a much larger number of cells (Fig. 521). In addition to fibroblasts and macrophages, lymphocytes of various types and plasma cells are present here in considerable numbers. In man granular leukocytes are rare and the presence of a noticeable number of them is always an indication of abnormal, inflammatory changes which have induced the migration of these elements from the blood vessels. In abnormal function of the mammary gland, the penetration of a very few wandering elements into the glandular epithelium and inside the alveoli can be observed.

Regression of the Mammary Gland. With the end of the nursing period, a regression takes place in the mammary gland. The glandular elements return to a resting state. The production of milk ceases, the secretion remaining in the glands is absorbed rapidly, an apparent increase in the stroma begins and the alveoli, due to a decrease in size and the degeneration of the glandular cells, diminish greatly and lose their lumen. The gland, however, does not return to its original state because many of the alveoli which had formed during the period of pregnancy do not disappear entirely, and the remains of the secretion may sometimes be retained in the mammary ducts for a considerable time. In such a resting condition the gland remains until the following pregnancy, when the same cycle of changes is repeated.

Involution of the Mammary Gland. In old age the mammary gland gradually undergoes involution; the epithelium of the secretory portions, and partly also of the excretory ducts, atrophies, and the gland tends, in a general way, to return to the prepubertal condition in which there are only a few scattered ducts. On the other hand, the epithelium is not infrequently the seat of a pathologic growth.

Equally striking changes occur in the interstitial connective tissue. This becomes decidedly less cellular; the number of collagenous fibrils decreases and the whole mass becomes more homogeneous and stains much less intensely with eosin. One author has pictured the change as having occurred through a "melting down" of the fibrillar intercellular tissue into a homogeneous mass. When stained with aniline blue, the interstitial substance in such a breast appears finely granular and deep blue.

Blood and Lymphatic Vessels. The blood supply of a functioning mammary gland is much greater than that of a resting gland The arteries arise from the internal mammary artery, the thoracic branches of the axillary artery and from the intercostal arteries. They pass mainly along the larger ducts and break up into very dense, capillary networks on the external surface of the basement membrane of the secretory portions (Fig 521). The veins empty into the axillary and internal mammary veins.

The lymphatic vessels begin with capillary networks located in the connective tissue layers surrounding separate alveoli. They collect along the course of the mammary ducts into a subpapillary lymphatic network; from here several large vessels lead the lymph mainly into the lymphatic nodes in the axilla, but also into connection with the lymphatics leading beneath the sternum, and even into those of the other breast.

Nerves. Besides the nerves which supply the smooth muscles of the blood vessels and of the papilla, the mammary gland has also secretory nerve endings closely connected with the glandular elements as well as rather numerous sensory nerve endings of the nipple; some of the latter have a structure similar to that of the genital bodies.

Histogenesis of the Mammary Gland. The primordium of the mammary gland appears in a human embryo of 8 mm. as a paired thickening of the epidermis, the "milk line," which begins at the root of the upper extremity and proceeds to the inguinal fold. It continues to thicken and becomes the *mammary fold;* this is retained in man in only a limited region of its cranial portion as a pair of flat, lens shaped thickenings. These become hemispherical or club-shaped, epithelial thickenings directed toward the underlying connective tissue This, in turn, thickens and lifts the developing gland slightly above the rest of the surface of the organism (in human embryos 19 to 30 mm. long). In most mammals several pairs of such thickenings are formed

The cells of this epithelial bud are cylindrical in shape and are arranged radially, while the deeper layers are polyhedral. By continuing to multiply they form, in human embryos of 50 to 60 mm, on the lower convex surface of the body, the primordia of 16 to 25 projections, with swellings at their ends; these projections gradually elongate in the direction of the connective tissue layer and become the mammary ducts. Their number fluctuates with the age of the embryo as they do not all originate at once; for this reason their lengths, at any given moment, are not equal The peripheral cells at first maintain their cylindrical shape while the others are multangular and have round nuclei. Externally they are gradually covered by a condensing connective tissue. In the course of time each of

these cylindrical, epithelial projections or shafts gives rise, by elongation and twisting, to larger and larger numbers of branches; these are also swollen at their ends and are the primordia of the *future excretory ducts. A lumen appears in them* except in the terminal swellings, due to the moving aside and to partial degeneration of the constituent cells. *Some of the cells which touch the connective tissue develop into basket cells of so-called "myo epithelial" nature.*

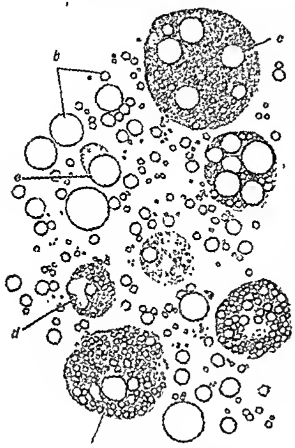

Fig. 523 Human colostrum; fresh preparation: *b*, Milk globules of various sizes; *c*, colostrum bodies with fat droplets of various sizes; *d*, colostrum bodies with nuclei; *e*, milk globules with "caps." 1000 ×.

In newborn individuals of both sexes, the glands have a diameter of 3.5 to 9 mm. and at this time contain a number of distinct alveolar portions, some of which, however, are very rudimentary. At the same time, in the lumen of the developing and branching ducts, a substance is formed which suggests colostrum, this secretion is called *witch's milk* and contains little but degenerating, fatty, epithelial cells; this secretion can be squeezed out of the papilla in newborn infants; it soon disappears

In males the mammary gland undergoes a regression and only the nipple remains with the surrounding areola. In females, however, the development, although rather slow, continues and *the slow elongation and branching of the epithelial shaft goes on throughout childhood.* With the onset of sexual maturity, the development *does not change qualitatively, but increases in intensity and quantity; each original epithelial shaft forms numerous branches through the multiplication of its constituent cells. After having* reached a certain degree of development the organ undergoes but slight changes and remains in a state of functional rest; there is no secretion and even the secretory portions are missing, according to most authors, who believe that only excretory ducts are present at this time.

Histophysiologic Remarks. Milk is an aqueous solution of albumins (mainly casein), lactose, and inorganic salts (including small amounts of iron) in which numerous fat drops, the *milk globules*, are *suspended. Some of these are at the limit* of visibility; most of them are 2 to 5 μ and seldom up to 10 to 12 μ in diameter. These are the same fat drops which are produced and given off by the glandular cells. Small numbers of milk globules with one-sided caps, free disintegrating nuclei, nucleated or non-nucleated fragments of glandular cells, and transformed leukocytes are also found.

All these constituents of the milk can be seen in histologic preparations in the lumens of the dilated secretory portions and of the excretory ducts; this is particularly clear after fixation with osmic acid, when the milk globules are stained black. The number of the cellular fragments and leukocytes appears to be larger in the alveoli than in the excreted milk; this shows that these structures autolyze during the excretion of milk.

During the last days of pregnancy, the *first two or three days after delivery*, and at the end of the period of lactation, the secretion of the mammary gland is quite different from milk and is called *colostrum* (Fig. 523). It differs from milk by *being poor in fat. But it contains numerous globules with particles of cellular* fragments, free nuclei and *colostrum bodies* (9 to 40 μ in diameter). These globu-

lar bodies are often capable of active ameboid movements. After fixation and staining they appear as free, large cells with usually one constricted nucleus; their cytoplasm is filled with many small and a few large fat drops (Fig. 523). A considerable portion of these elements show indications of degeneration.

Many authors recognize the colostrum bodies as transformed epithelial glandular cells which are filled with the products of secretion and have become detached. But it is more probable that they are wandering lymphoid cells of various kinds, which have escaped from the connective tissue into the epithelium and into the lumen of the glandular spaces, and which ingest fat droplets by phagocytosis. During the periods when colostrum appears, the interstitial connective tissue which surrounds the secretory portions is heavily infiltrated by hematogenous and histogenous lymphocytes. In fixed preparations these elements may often be seen on their way through the basement membrane into the epithelium and thence into the lumen of the alveolus.

The colostrum bodies always appear when the equilibrium between the secretion of the milk and its excretion is upset, that is, either when the feeding of the child has not yet begun, as in the last days of pregnancy, or when it has come to an end. The presence in the colostrum of hematogenous granular leukocytes, particularly of the neutrophil type, is usually an indication of an inflammatory process in the mammary gland.

The proliferative changes in the gland during gestation are due mainly to hormones arising in the ovary (estrone and progesterone) apparently acting through the pars distalis of the hypophysis. for these hormones have no effect on the mammary gland in hypophysectomized animals. A mammogenic duct growth factor has been extracted from the pars distalis of the hypophysis. The initiation of lactation seems to be due to the lactogenic hormone of the pars distalis of the hypophysis. The factors involved in lactation are discussed by Petersen.

The production of the colostrum in newborn infants probably depends on the same hormones which bring about the production of milk in the mother. Some believe that there are indications of a dependence of this process on the hypophysis of the embryo.

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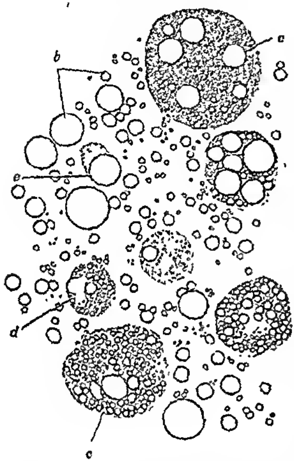

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the ocular tissues. The larger posterior portion of the uvea extends anteriorly as far as the ora serrata, and is called *chorioidea* or *chorioid membrane*. Its anterior thickened, muscular portion is the *ciliary body* which forms a girdle from 5 to 6 mm. wide between the ora serrata and the sclerocorneal junction. This body is the muscular instrument for the accommodation of the refraction of the eye. The thin, curtain-like membrane, the *iris*, is the further continuation of the uvea. Its outer brim is attached to the ciliary body. The iris projects into the cavity of the eye in a plane approximately vertical to the visual axis. Its inner margin slides over the anterior surface of the crystalline lens. The diameter of the iris is approximately 12 mm. Its central opening, the *pupil*, can be reduced or expanded through the contraction or relaxation of the constrictor and the dilator muscles of the pupil. In this way the iris functions as an adjustable optic diaphragm regulating the amount of light entering the eye.

The third or innermost tunic, the *retina* (Fig. 524) contains in its posterior functioning part (pars optica retinae) cells that are sensitive to light or photoreceptors (rods and cones), and others that are the first links of the nervous pathway conveying the nervous impulses through the optic nerve to the brain. The spot where the nerve inserts into the eyeball, the *papilla of the optic nerve*, is a round or somewhat elliptical, pink disk measuring approximately 1.4 mm. across. It is situated at a distance of about 3 mm. nasal to the posterior pole of the eye, and somewhat more from the center of the fovea. The portion lining the inner surface of the ciliary muscle, the *ciliary portion of the retina*, and that lining the posterior surface of the iris, the *iridial portion of the*

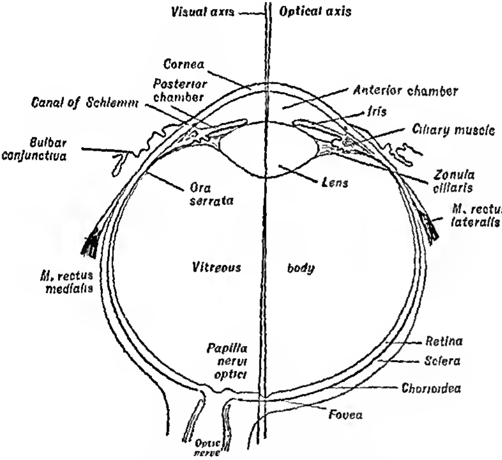


Fig. 524. Diagram of a horizontal section through the right eye of man.

Chapter XXVII

THE EYE*

THE ability to react to light is a widespread property of living matter. Yet in order to react to light in a specific way, certain cells of the body have increased this general responsiveness to light. Scattered photoreceptive cells in lower animals probably distinguish only various intensities of light, and, at best, only crudely perceive the direction of the light stimuli. In higher animal forms, especially in vertebrates, more efficient organs have evolved which react not only to various intensities and qualities of light, but are capable of distinguishing the form, size, and minute changes in the position of external objects.

The eyes of vertebrates have evolved from the central nervous system. Two symmetrical localities, one on each side of the front end of the brain, became especially photosensitive. Out of these the photoreceptive organs, the retinae of the two eyes, developed.

The retinae are thin membranes of nervous substance, resembling the brain in structure. They are stretched out in such a way as to catch the rays of light arriving from various directions. Since light propagates in straight lines and is refracted by curved surfaces, a system of transparent media evolved which permits the distribution of the rays of light upon the retinal surface according to the laws of physical optics. Moreover, the tunics and intrinsic and extrinsic muscles were added; their chief functions are to keep the dioptric system of the eye in a definite relation to the photosensitive retina, and to change the refraction of the media or the position of the eyes, as occasion requires. In addition, the eye is supplied with blood vessels, nerves and glands, and is located in the bony orbit of the skull where it can be covered by the lids.

STRUCTURE OF THE EYE IN GENERAL

The structural principle of the human eye is much the same as in the photographic camera (Fig. 524). The anterior segment of its wall, the cornea, is transparent and permits the rays of light to enter. The rest of the wall of the eye is opaque. It has a darkly pigmented inner surface which absorbs light rays, and is to a great extent lined with the photosensitive retina. The cavity of the eyeball is filled with transparent media arranged in separate bodies. Their curved surfaces, including those of the cornea, are so formed and strung along a straight line, the optic axis, as to act as a system of convex lenses. These produce an *inverted*, reduced and real image of the objects of the outside world in the photoreceptive layer of the retina, the rods and cones (2 in Figs. 534, 535, 536, 539).

The wall of the eyeball is composed of three coats (Fig. 524). The thick and tough outer *fibrous tunic* gives the organ its form and protects its inner delicate structures. It is subdivided into a large opaque posterior portion called *sclera*, and a smaller anterior transparent segment, the *cornea*. The middle *vascular tunic* or *uvea* is rich in blood vessels and is chiefly concerned with the nutrition of

* This chapter has been rewritten by S. Polyak.

or nasal and an outer or temporal half. The plane of the horizontal meridian divides the eyeball and retina into an upper and a lower half. Together the two planes or meridians, the sagittal or vertical and the horizontal, divide the eyeball and the retina into four quadrants, an upper nasal and an upper temporal, a lower nasal and a lower temporal. The point where the two planes cross in the eye fundus, that is where the visual axis passes through the foveal center, corresponds with the *point of fixation* in the field of view, or the point where the eye is arrested in looking at an object, corresponding with the spot of most distinct vision.

The largest sagittal or *antero-posterior diameter* along the axis of the eye is 24 mm., or a little more. It is identical with the *outer or external axis* of the eye which includes the thickness of the walls. The *inner axis* is the largest sagittal distance between the inner surface of the cornea and the inner surface of the retina at the posterior pole: it measures a little less than 22 mm. The *optical axis* (Fig. 524) is a sagittal line that passes through the optical centers of the refractive media. Because of this the eye can be regarded as a centered optical system. The optical axis is almost identical with the anatomical and geometrical axes. It differs, however, from the *visual axis*. The latter where it touches the fundus passes through the center of the fovea. The visual axis thus deviates from the optical axis at the posterior extremity where it touches the retina; it is from 4 to 7 degrees temporal (lateral) and 3.5 degrees below the optical axis. The *vertical axis* is a perpendicular line connecting the opposite points of the equator and passing through the axis of the eye; it measures approximately 23.5 mm. A similar horizontal line, the transverse axis, measuring 24 mm. or somewhat more, represents the greatest diameter of the eyeball.

The *shape of the eyeball* is partly due to the anatomical structures, and is partly the product of the intra-ocular pressure maintained by the pressure of blood and other fluids. Since the curving of its wall varies somewhat in different localities, the shape of the eyeball is not that of a perfect sphere. Thus the *radius of the curvature* of the larger posterior segment around the fundus is the largest, measuring somewhat less than 13 mm., gradually decreasing toward the corneoscleral junction. The cornea has the smallest radius of curvature, approximately 7.8 mm. (outer corneal surface). In the horizontal plane, the radius of curvature of the eyeball is somewhat larger in the nasal than in the temporal half. Additional deformation is produced by the pressure of the several extrinsic eye muscles.

The eyeball is lodged in a soft, elastic cushion filling the bony orbit of the skull and made up of loose connective and fatty tissue, muscles, fasciae, blood, and lymphatic vessels, glands and nerves. This permits the eye to move freely around its *center of rotation* (which, however, itself is not quite stable, but is somewhat dislocated with each movement of the eyeball). With the general integument the eye is connected by the conjunctiva. The lids are a mechanical protection against external noxious agents.

THE FIBROUS TUNIC

The Sclera. The thickness of the sclera at the posterior pole of the eyeball reaches 1 mm. At the equator it is reduced to 0.4 to 0.3 mm., while toward the edge of the cornea it again increases to 0.6 mm. The sclera (Figs. 525, *SK*; 527 and 529, *S*; 533, *d*) consists of flat collagenous bundles which run in various directions parallel to the surface. The tendons of the eye muscles are attached to the outer surface of the sclera. Between these bundles are fine elastic nets. The cells of the sclera are flat, elongated fibroblasts. In the deeper layers, especially in the region of the entrance of the optic nerve, a varying number of chromatophores can be found.

The outer surface of the sclera is connected by a very loose system or thin collagenous bundles and membranes separated by cleftlike spaces—the *space of Tenon*—from a dense layer of connective tissue, the *capsule of Tenon*. This arrangement makes rotating movements of the eyeball possible in all directions.

Between the inner surface of the sclera and the chorioid there is a layer of loose connective tissue with numerous branched chromatophores, fibroblasts, and elastic networks. This tissue anatomically belongs to the chorioid, but when these tunics are separated, it tears and remains attached partly to the sclera and partly to the chorioid. The first part is sometimes called the *lamina fusca of the sclera*, because of its brown color, or the *suprachorioid lamina* (Fig. 525, *sch*).

Cornea. The cornea is slightly thicker

retina, are not photosensitive and are therefore called the blind portion of the retina.

The space enclosed by the tunics of the eye is filled with the transparent *dioptric media* (Fig. 524). With these, however, the *cornea* must be included. Because of the considerable difference between the index of refraction of the cornea (1.376) and of the surrounding air (1.0), the cornea is the chief refractive apparatus of the eye. Of the enclosed transparent media the most anterior is the *aqueous humor* (p. 629). It is contained in the *anterior chamber*, a small cavity bordered in front by the cornea and behind by the ciliary body, the iris and the central portion of the anterior surface of the lens. Its depth is greatest in the region of the optic axis (3.10–3.60 mm.), gradually decreasing toward its periphery; it measures 12 mm. across. Its outermost portion is at the "angle of the iris." The *posterior chamber* is a narrow circular space enclosed by the iris, the lens, the ciliary and the vitreous bodies (Figs. 524, 529). It also comprises the spaces between the fibers of the ciliary zonule. The prezonular space, or the posterior chamber in the strict sense, is the above-described space minus the spaces between the zonular fibers. Its greatest depth is from 0.4 to 0.6 mm. The remainder of the posterior chamber is made up of the circumlental space whose greatest depth is 0.5 mm. (*Cl* in Fig. 528), and of the orbicular space or the cleft between the ciliary ring and the anterior limit of the vitreous. Both chambers are filled with aqueous humor. This is a clear, watery fluid of slightly alkaline reaction with an index of refraction of 1.33. It contains 0.77 per cent sodium chloride, traces of urea and glucose, and practically no proteins, and few or no wandering cells. It is much like the cerebrospinal fluid.

The next of the transparent media is the *crystalline lens* (Fig. 524). This is an elastic biconvex body suspended from the inner surface of the ciliary body by a circular ligament, the ciliary zonule. It is placed directly behind the pupil, between the aqueous humor of the anterior chamber and the cornea anteriorly, and the vitreous body posteriorly. The lens is second in importance to the cornea as a refractive apparatus of the eye, and is the dioptric organ of accommodation.

The posterior larger portion of the cavity of the eye, between the posterior surface of the lens, the ciliary body, and the posterior wall of the eyeball, called *vitreal cavity*, is filled with a thick, viscous, semifluid substance, the *vitreous humor or body*, or, briefly, vitreous. It immediately adjoins the retina and thus enables light to pass freely to the photoreceptors.

The retina, too, during life possesses an almost ideal transparency. Only its outermost layer, immediately bordering the chorioid, and made up of the pigment epithelium (1 in Figs. 524, 525, 529) is opaque and is the first barrier to the rays of light.

MEASUREMENTS AND LANDMARKS

The adult human eyeball is a fairly regular spherical body measuring approximately 24 mm. or 1 inch in diameter. Its weight differs individually from 6 to 8 gm. Its anterior extremity corresponding with the center or vertex of the cornea is the *anterior pole*. Its posterior extremity corresponding with the farthest point of the fundus, located between the central fovea and the optic papilla, is the *posterior pole*. The line connecting the two poles is the anatomical axis which is practically identical with the *geometrical axis* of the eye. The *visual axis* is the line drawn from the point of fixation (center of the fovea) to the apparent center of the pupil. A circular line whose plane, the equatorial plane, is vertical with respect to the axis and passes through the greatest expansion of the eyeball is the *equator*. Other similar circles but with planes passing through the axis or, meaning the same, through the two poles, are the *meridians* of the eye. The two most important are the vertical and the horizontal meridians. The sagittal plane of the first passes through the fovea and divides the eyeball, including the retina, into an inner

is a mucoid cement-like substance. In vitally stained, newborn animals, the corneal cells store a few dye granules.

Besides the fibroblasts, the substantia propria always contains a number of lymphoid wandering cells which migrate from the blood vessels of the corneal limbus. In inflammation the tissue is infiltrated with enormous numbers of heterophil leukocytes and lymphoid cells. Penetrating between the lamellae, they become arranged in typical fusiform rows. The substantia propria contains very fine elastic networks especially abundant in the innermost layers in front of the membrane of Descemet. The usual stains for elastin do not always demonstrate them.

Membrane of Descemet. This is a homogeneous lamella 5 to 10 μ thick (Fig. 526, *Ep*). It can be isolated from the posterior surface of the substantia propria. At the periphery of the cornea the membrane of Descemet continues as a very thin layer on the surface of the trabeculae of the iridial angle.

Although it is easily stained with resorcin-fuchsin and other dyes, its reactions are different from those of typical elastin. It is a basement membrane which is believed to be secreted by the corneal endothelium. After injuries it is said to regenerate through the activity of the latter.

Corneal Mesenchymal Epithelium. The inner surface of the membrane of Descemet is composed of one layer of large (18 to 20 μ), squamous cells (Fig. 526, *En*). In man their flattened nuclei are round or oval.

Sclerocorneal Junction. In a meridional section the boundary between the white, opaque sclera and the transparent cornea appears as an oblique line (Fig. 529, *SF*). The outer edge of the sclera (*S*) overlaps the border of the cornea (*K*), while on the inner side the cornea extends farther backward. When the collagenous bundles of the sclera continue directly into those of the cornea, their striation loses its distinctiveness and the tissue becomes homogeneous and transparent.

The marginal zone of the cornea, measuring about 1 mm. in width and outlined centrally by the edge of the membrane of Bowman, is called the *edge or limbus of the cornea.* In this region the epithelium of the cornea gradually changes into that of the conjunctiva of the bulb (Fig. 529, *C*). Where the membrane of Bowman ends, a subepithelial layer of loose connective tissue begins; it contains the superficial, marginal loops of the vessel, which are of special importance because they furnish the nutritive material

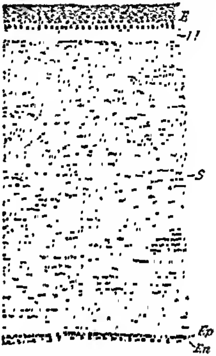


Fig. 526. Vertical transection of the middle part of a human cornea: *E*, Epithelium; *El*, Bowman's membrane; *S*, substantia propria; *Ep*, membrane of Descemet; *En*, Corneal mesenchymal epithelium. 135 ×. After Schaffer.

to the cornea and also are the source of the wandering cells just mentioned. The blood vessels which invade the substantia propria in chronic inflammation also arise from these loops.

On the inner surface of the wall of the eyeball the sclerocorneal junction is marked by a shallow, ring-shaped furrow, the *internal scleral furrow or sulcus.* Its posterior lip forms a small centrally projecting ridge, the *scleral roll* to which the ciliary body is fastened (Fig. 527, *Sw*). At the bottom of the internal scleral furrow the scleral tissue contains one or several cavities lined with endothelium. They are the cross sections of a circular canal which parallels the border of the cornea and in many places breaks up into several irregular branches which then fuse again. It is the *canal of Schlemm*, or the "venous sinus" of the sclera (Figs 527, *Sch*; 527, *Sc*). It communicates with the venous system and is believed to drain the aqueous humor from the an-

than the sclera and measures 0.8 to 0.9 mm. in the center and 1.1 mm. at the periphery. In man the refractive power of the cornea, which is a function of the index of refraction of its tissue (1.376) and of the curvature of its free outer surface (7.8 mm.), is twice as high as that of the lens.

In a vertical section through the cornea, the following layers can be seen: (1) the epithelium, (2) the membrane of Bowman, (3) the stroma or the substantia propria, (4) the membrane of Descemet, (5) the corneal mesenchymal epithelium (Fig. 526).

Epithelium. The epithelium is of the stratified squamous type with an average thickness of 50 μ and consists, as a rule, of five layers of cells (Fig. 526, *E*). The basal cells, adjacent to the membrane of Bowman, are large cuboidal or low columnar elements with a rounded outer surface, a clear cytoplasm, a round or oval nucleus, and a diplosome in the apical part of the cell body. The cells of the second layer have an irregular polyhedral form. In the successive layers the cells become increasingly flattened. The outer surface of the cornea is quite smooth and is composed of large squamous cells.

As in other types of stratified squamous epithelium (p. 31) the cells in the corneal epithelium are connected with one another by thin intercellular bridges. Very often wandering lymphoid cells penetrate the spaces between the cells where they appear as irregular, branched elements, the cells of Langerhans; they come from the blood vessels at the periphery of the cornea.

The epithelium of the cornea is extremely sensitive and contains numerous free nerve endings (Fig. 180). It is endowed with a remarkable capacity for regeneration. Small defects of the epithelial layer, caused by injuries, heal rapidly by a flattening and gliding movement of the adjacent epithelial cells. Mitoses in the basal epithelial cells set in later and may be found at considerable distances from the wound. In normal conditions a few mitoses can be found in the basal cell layer.

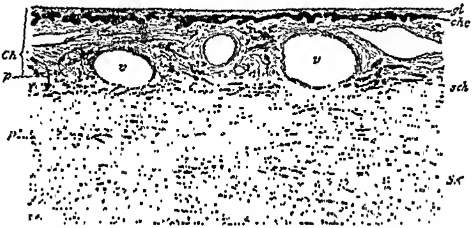


Fig. 525. Chorioid and sclera of an enucleated eye in cross section: *Ch*, Chorioid; *SK*, sclera; *chc*, choriocapillary lamina; *gl*, glassy membrane; *p*, pigment cells (melanoblasts); *p'*, pigment cells in the sclera; *sch*, suprachoroid lamina; *v*, veins. 135 ×. After Schaffer.

Bowman's Membrane. The corneal epithelium rests upon the 6 to 9 μ thick, indistinctly fibrillated membrane of Bowman. This is a condensed outer layer of the subjacent substantia propria from which it cannot be isolated (Fig. 526, *El*). At the periphery of the cornea it ends abruptly. The membrane does not contain elastin.

Substantia Propria. This layer (Fig. 526, *S*) forms about 90 per cent of the cornea. It is a transparent, regular connective tissue whose bundles form thin lamellae arranged in many layers. In each layer the direction of the bundles is parallel; in the adjacent lamellae the bundles intercross at various angles. The lamellae everywhere interchange fibers and thus are kept tightly together. Between the fibrils, the bundles and the lamellae, there

serrata. This layer contains flattened fibroblasts, which by some authors were wrongly described as a separate endothelial layer.

Glassy or Bruch's Membrane. This is a brilliant limiting line 1 to 4 μ thick between the chorioid and the pigment epithelium of the retina (Fig. 525, *gl*). It can be subdivided into two lamellae. The outer one, facing the capillary layer, is formed by a dense plexus of finest elastic fibers which are the continuation of the elastic nets of the capillary interstices. The inner, thicker lamella is homogeneous. Whereas the first is of connective tissue origin, the second is produced by the pigment epithelium of the retina.

Ciliary Body. If the eyeball is cut across along its equator, and its anterior half, after removal of the vitreous, is inspected from within, a sharply outlined, dentate border is seen running around the inner surface of the wall in front of the equator. This is the *ora serrata* of the retina (Fig. 528, *Os*). The girdle between the ora and the edge of the lens (*L*) is the *ciliary body*. It is formed by a thickening of the vascular tunic of the eye. Its inner surface is covered by the darkly-pigmented ciliary portion of the retina. In a meridional section through the eye bulb, the ciliary body appears as a long, thin triangle with its small base facing the anterior chamber of the eye and attached here by its outer angle to the scleral roll; the long narrow angle extends backward and passes into the chorioidea (Fig. 529).

The inner surface of the ciliary body is subdivided into a narrow anterior zone, the *corona* or *ciliary crown* (Fig. 528, *Cor*), and a broader posterior zone, the *orbicular* or *ciliary ring* (Fig. 528, *Or*). The inner surface of the ring is fairly smooth and shows only shallow meridional grooves, ciliary striae, which run forward from the teeth of the ora.

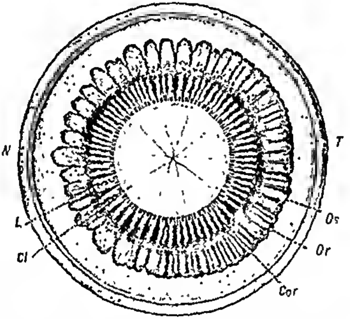


Fig. 528 Anterior half of the eye seen from within: *N*, Nasal and, *T*, temporal side; *Os*, ora serrata retinae, *Or*, ciliary ring, *Cor*, ciliary crown; *Cl*, circumlental space; *L*, posterior surface of the lens with the lens star 3 × After Salzmann.

The ciliary crown has a uniform width along its entire circumference. It carries on its inner surface seventy radially arranged ridges or folds, the *ciliary processes* (Fig. 529, *PC*). The crests of the ridges are less pigmented than their lateral surfaces and the depressions between the ridges.

Ciliary Muscle. The main mass of the ciliary body, exclusive of the ciliary proc-

terior chamber (see p. 629). It is usually filled with clear liquid and contains blood only in cases of stasis in the venous system.

The *transparency of the cornea*, its most remarkable property, though less than that of the aqueous humor and of glass, is high. It is due partly to the great regularity of its structural composition, and partly to other factors still improperly understood: chemical, microphysical (colloidal), and others.

THE VASCULAR TUNIC (UVEA)

Chorioid Membrane. The chorioid is a thin, soft, brown membrane adjacent to the inner surface of the sclera (Fig. 525, *Ch*). Between the sclera and the chorioid is a potential cleft, the perichorioidal space; it is traversed by thin lamellae which run obliquely from the chorioid to the sclera and form a very loose, pigmented tissue layer—the suprachorioid layer.

The *suprachorioid layer* is composed of fine, transparent membranes or lamellae with fibroblasts on their surface and with a rich network of elastic fibers (Fig. 525, *sch*). Everywhere between and in the lamellae large flat melanoblasts are scattered (Fig. 525, *p*). In the suprachorioid as in the rest of the uvea are scattered macrophages.

The lamellae of the suprachorioid pass without a distinct boundary into the substance of the chorioid. This tunic can be subdivided into three main layers. From outside inward they are: (1) the vessel layer, (2) the capillary layer, and (3) the glassy or Bruch's membrane.

Vessel Layer. This layer consists of a multitude of intercrossing large and medium-sized arteries and veins (lower portion of *Ch* in Fig. 525). The spaces between the vessels are filled with loose connective tissue rich in chromatophores. The lamellar arrangement here is much less distinct than in the suprachorioid. According to some, the vessel layer contains strands of smooth muscle which are independent of the arteries.

Choriocapillary Layer. This is formed by a capillary network arranged in one plane (*chc* in Fig. 525). In places this layer is connected with the vascular layer. The individual capillaries have a large and somewhat irregular caliber. The net is especially dense and the capillary layer much thicker at the posterior pole of the eyeball, in the region of the fovea. Anteriorly it ceases in the region of the ora

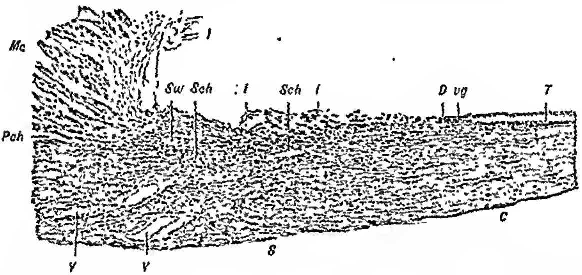


Fig. 527. Meridional section through the scleral furrow: *C*, Cornea; *D*, border of Descemet's membrane; *I*, iris root; *i*, uveal framework; *Mc*, ciliary muscle; *Pch*, beginning of the perichorioidal space; *Sch*, canals of Schlemm; with their veins; *V*; *Sw*, scleral roll; *T*, deep root of the scleral framework; *vg*, anterior marginal ring. 96 ×. After Salzmann.

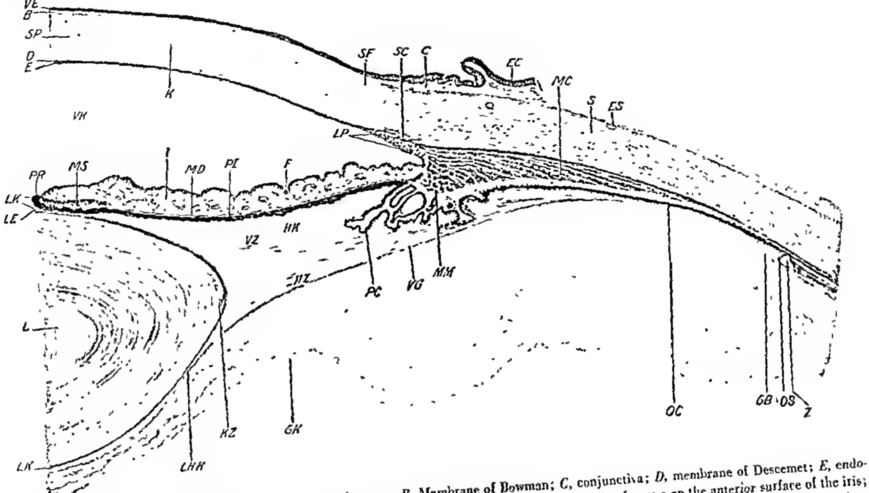


Fig. 529. Part of a meridional section of the eyeball of a man: *B*, Membrane of Bowman; *C*, conjunctiva; *D*, membrane of Descemet; *E*, endothelium of the cornea; *EC*, stratified epithelium of the conjunctiva, *ES*, episcleral tissue; *F*, contraction furrows on the anterior surface of the iris; *GB*, base of the vitreous; *GK*, fibers of the vitreous; *HK*, posterior chamber; *HZ*, posterior fibers of the *zonule*; *I*, iris; *K*, cornea; *KZ*, nuclear zone of the lens; *L*, nucleus of the lens; *LE*, epithelium of the lens, *LHK*, hyaloideo capsular ligament; *LK*, capsule of the lens; *LP*, ligamentum pectinatum; *MC*, ciliary muscle; *MD*, dilator pupillae; *MM*, circular fibers of the ciliary muscle; *MS*, sphincter pupillae; *OC*, orbiculus ciliaris; *OS*, ora serrata; *PC*, ciliary processes; *PI*, pars iridica retinae; *PR*, border of the pupil; *S*, sclera; *SC*, venous sinus of the sclera (canal of Schlemm); *SF*, edge of sclera overlapping the cornea; *SP*, substantia propria of the cornea; *VE*, corneal epithelium; *VG*, anterior limit of the vitreous; *VK*, anterior chamber, *VZ*, anterior fibers of the ciliary zonule; *Z*, cystic cavities in the retina. 17 ×. After Schaffer.

esses, consists of the smooth *ciliary muscle* (Fig. 529, *MC*). It occupies a peripheral position, and, in a meridional section, repeats the triangular form of the ciliary body. It can be considered as the anterior, thickened edge of the suprachorioid lamina attached to the sclerocorneal junction.

The ciliary muscle is composed of three portions. The outermost, closest to the sclera, is the muscle of Brücke whose bundles form a dense, netlike framework stretched chiefly in the meridional direction. Anteriorly this portion increases in thickness until it constitutes about one third of the ciliary muscle. It is partly inserted to the scleral roll, and partly directly attached to the sclera (Crampton's muscle). This *outer part of the ciliary* muscle stretches the chorioidea and is also called *tensor muscle of the chorioid.* Its rôle in the process of accommodation is discussed in the section dealing with the lens. In the next inward portion of the ciliary muscle, in its deeper layers, the bundles of muscle cells radiate fanlike from the region of the scleral roll toward the cavity of the eyeball; this is the *radiated or reticular portion of the ciliary muscle.* The inner angle of the ciliary muscle, in a meridional section, consists of cross sections of small bundles. This is the third or circular portion of the ciliary muscles, or the *muscle of Müller* (Fig. 529, *MM*). This portion is usually absent in the newborn, appearing in the course of the second or third year.

The fibers of the ciliary muscle contain an oval nucleus and very distinct myofibrils. The cleftlike meshes between the muscular bundles are filled with a small amount of connective tissue with abundant elastic networks and chromatophores. The latter become especially numerous toward the sclera and backward, where the connective tissue gradually passes into the lamellae of the suprachorioid.

The inner, *vasculor layer of the ciliary body* consists of connective tissue with numerous blood vessels. In the ciliary ring it is the direct continuation of the same layer of the chorioid. In the region of the ciliary crown it covers the inner surface of the ciliary muscle and forms the core of the ciliary processes. The vessels are almost exclusively capillaries and veins of varying caliber which have mostly a meridional course. The corresponding arteries branch in the peripheral layers of the ciliary body.

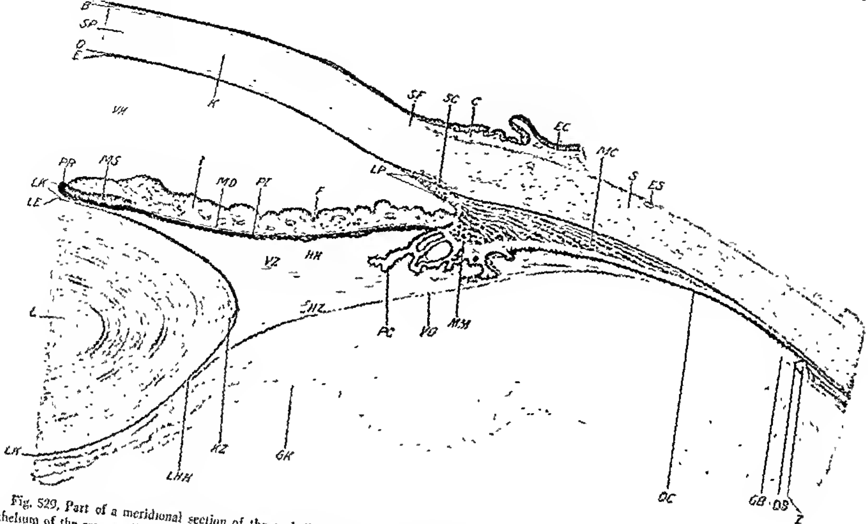
The connective tissue is dense, especially near the *root of the iris,* and contains abundant elastic networks. In old age it often shows hyaline degeneration. Chromatophores are usually found only *near the surface of the muscle.*

The inner surface of the vascular layer of the ciliary body is lined by the continuation of the *glassy membrane* of the chorioid. *In the region of the ciliary body,* however, this membrane splits into three distinct lamellae, where a thin additional intermediate layer is interposed between the two lamellae seen in its chorioidal portion.

Immediately adjacent to the connective tissue of the vascular layer of the ciliary body is the elastic lamella, a thin, dense, elastic network. Inwardly it is followed by a dense, meridionally fibrillated collagenous sheet with a few fibroblasts. Finally, the inner surface of the uveal portion of the ciliary body with its striae and excrescences is coated with the cuticular lamella. The epithelial layer is attached to its wrinkled surface.

The *ciliary portion of the retina* continues forward beyond the ora serrata, where it covers the inner surface of the ciliary body. This part consists merely of an outer pigmented layer and of a nonpigmented inner layer, and does not play any rôle in the perception of light stimuli.

This deeply pigmented epithelium consists of one layer of columnar cells and



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A thin layer of the stroma *immediately* subjacent to the mesenchymal epithelium, the anterior stromal sheet or lamella, is devoid of blood vessels. Farther inward is the thick vessel layer. Its posterior surface is covered with a double layer of heavily *pigmented epithelium*, the iridial portion of the retina.

Anterior Stromal Sheet or Lamella. This contains, in a homogeneous *ground* substance, a few collagenous fibers and a large number of polymorphous fibroblasts and *numerous chromatophores*. The color of the iris depends on the quantity, the color, and the arrangement of the pigment and on the thickness of the lamella.

If this layer is thin and its cells contain but little or no pigment, the black pigment epithelium on the posterior surface, as seen through the semiopaque, colorless tissue, gives the iris a blue color (Fig. 530, *B*). An increasing amount of pigment brings about the different shades of gray and greenish hues. Large amounts of dark pigment cause the brown color of the iris (Fig. 530, *A*). *In albinos the pigment is absent or very scanty* and the iris is pink because of its blood vessels.

Vessel Layer. *This layer contains* the numerous blood vessels. The spaces between them are filled with a loose, spongy connective tissue. Among the branched chromatophores in the pupillary zone, in front of the sphincter of the pupil, a variable number of large, round pigment cells is scattered. These cells are believed to be displaced cells of the pigment epithelium of the iridial portion of the retina.

Iridial Portion of the Retina. The epithelial pigment layer on the posterior surface of the iris genetically represents the anterior section of the secondary optic vesicle from which the retina *originates*. It is the direct continuation of the ciliary portion of the retina and, like the *latter, originally* consists of two layers of epithelium. Beginning with the ciliary margin of the iris, however, these two layers assume a different character. The inner nonpigmented layer of the ciliary portion becomes heavily pigmented in the iridial region, and the outer pigmented layer becomes less pigmented. In the iris the outer less pigmented layer differentiates into smooth muscles (Fig. 530, *FL, FQ*).

Muscles of the Iris. Being an adjustable diaphragm, the iris contains two smooth muscles *which, through their tonus, keep this membrane* constantly stretched and press it against the surface of the lens. The contraction of the circular *sphincter of the pupil* reduces the pupil (Figs. 529, *MS*; 531, *Sph*). It is a thin, flat ring whose breadth changes according to the contraction of the iris, from 0.6 to 1.2 mm. It surrounds the margin of the pupil and is separated from the retinal pigment layer by a very thin, slightly condensed, connective tissue layer. Its smooth muscle fibers are arranged in thin circular bundles and on the posterior surface are often seen to course obliquely to the *dilatator of the pupil*.

The muscle which widens the pupil, the *dilator of the pupil*, consists of radially arranged myo-epithelial elements (Figs. 529, *MD*; 530, *FL*, 531, *Dil*). These form a thin membrane between the posterior surface of the vessel layer and the anterior surface of the pigment epithelium and arise through the transformation of the major part of the anterior or outer layer of the iridial portion of the retina.

The elements of the dilator at first are spindle-shaped, fibrillated cells with their cytoplasm moderately pigmented in the region of the nucleus. In the adult, however, the fibrillated parts of the cells become arranged as a separate, con-

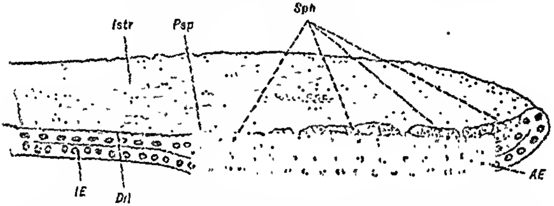

Fig. 531. Radial (meridional) section of the pupillary border of the iris of a newborn infant: *IE*, Inner epithelial layer and, *AE*, outer epithelial layer of the optic cup, *Sph*, sphincter; *Dil*, dilator of the pupil; *Psp*, spurlike process of epithelium, *Istr*, stroma of the iris. After v. Szily, from Franz.

continues upon the posterior surface of the iris where it partly undergoes a muscular differentiation. The inner, colorless layer is a simple columnar epithelium. The height of its cells decreases from behind forward. Its inner surface is lined with a distinct glassy membrane—the *ciliary inner limiting membrane.* It is a cuticular product of the epithelium and is considered to be a continuation of the inner limiting membrane of the optical portion of the retina.

Toward the root of the iris, on the anterior surface of the ciliary processes, the cells of the inner epithelial layer gradually begin to accumulate pigment granules. On the posterior surface of the iris they are as heavily pigmented as the outer layer. This is the *iridial portion of the retina* (Fig. 529, *PI*).

Iris. The posterior surface of the iris near the pupil rests upon the anterior surface of the lens; in this way the iris separates the anterior chamber from the posterior (Fig. 529, *I*). The peripheral margin of the iris, connected with the ciliary body, is called the *ciliary margin* or the root of the iris. The pupil is surrounded by the *pupillary margin of the iris* (Fig. 529, *PR*). In a meridional section the thickness of the iris diminishes toward both margins.

On examination in the living, the anterior surface of the iris presents, besides its individually varying color, certain distinct markings. At a distance of about 1.5 mm. from the pupillary margin a concentric, jagged line separates the anterior surface into an inner, pupillary, and into an outer, wider, ciliary zone. In the neighborhood of both the pupillary and the ciliary margins the anterior surface has many irregular excavations, the *crypts,* which may extend deep into the tissue. In addition, there are oblique, irregularly arranged, contraction furrows; these are especially marked when the pupil is dilated (Fig. 529, *F.*)

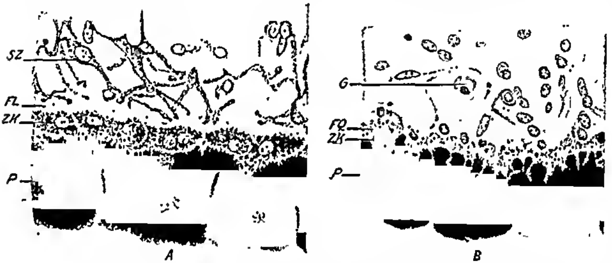

Fig. 530. *A,* Posterior part of a radial (meridional) transection of a dark human iris, from an enucleated eyeball: *FL,* Fibrillae of the dilator muscle in longitudinal section; *P,* pigment epithelium of the Inner (posterior) layer of the pars iridica retinae; *SZ,* pigment containing connective tissue cells (melanophores) of the vascular layer; *ZK,* pigment-containing cell bodies of the dilator muscle (outer or anterior layer of the iridial portion of the retina). *B,* Tangential, section of a light human iris. The fibers of the dilator muscle in cross section (*FQ*); *C,* blood vessels in the stroma. 380 ×. After Schaffer.

Being a part of the uvea and a continuation of the ciliary body, the iris in its main mass consists of a loose, pigmented, highly vascular connective tissue similar to the parts just mentioned. The anterior surface of this "stroma" is said to be lined with endothelium, which continues here from the posterior surface of the cornea.

muscle, there is always a simultaneous contraction of the pupillary sphincter.

Pigment Epithelium of the Iris. The large, prismatic cells of the pigment epithelium contain quantities of round, coarse, dark-brown melanin granules that render their outlines obscure and make their nuclei barely visible. At the edge of the pupil the pigment epithelium bends slightly around the border and is seen through the cornea as a thin, dark, pigmented seam outlining the aperture (Fig 529, *PR*). Bleached preparations show that at this place both original layers of the iridial portion of the retina (*IE, AE*) keep their epithelial character and turn into each other in the form of a fold (Fig. 531). The outer layer remains here in close contact with the *inner edge of the sphincter* muscle (*Sph*).

The posterior (inner) surface of the pigment layer is covered with a very fine membrane, the *limiting membrane of the iris,* a continuation of the inner limiting membrane of the ciliary portion of the retina.

Angle of the Iris. This is a circular recess at the periphery of the anterior chamber of the eye where the posterior surface of the cornea and the anterior surface of the iris meet (Figs. 529, 532). It seems to play an important rôle in the circulation of the intra-ocular liquid and shows a *peculiar interlacing of the constituents* of the two parts just mentioned. The elements of the sclera and of the ciliary muscle also take part in the formation of this region.

The endothelial wall of the *canal of Schlemm* (Figs. 527, *Sch;* 529, *SC;* 532, *S*) at the corneoscleral junction is surrounded by a layer of loose connective tissue with numerous cells. This is especially prominent at its inner periphery. Here a broad band of loose connective tissue trabeculae, originating from the posterior layer of the cornea and lined with endothelium, separates the canal of Schlemm from the anterior chamber. This meshwork is the *iris angle.* It extends from the edge of the membrane of Descemet to the scleral roll and to the root of the iris. Its meshes, the *spaces of Fontana,* are in direct communication with the anterior chamber. It is subdivided into a larger and coarser part, the *scleral framework* (Fig 527, *T*), which is adjacent to the sclera, and into a smaller, more delicate part, connected with the iris, the *uveal framework* or the *pectinate ligament of the iris* (Figs. 527, *t*; 529, 532, *Lp*). The trabeculae of the uveal framework are thinner, threadlike and do not contain any elastic elements. The meshwork receives small reinforcements from the connective tissue of the ciliary muscle and from the sclera. In the human eye the meshwork of the iris angle, especially its pectinate ligament, is much less developed than in some mammals.

THE RETINA

The retina is the innermost of the three coats of the eyeball (Fig. 524). Being the actual photoreceptor organ, it is the most important and at the same time the most complex structure of the eye. The retina arises in the early states of embryonic development through a bilateral evagination of the lateral walls of the prosencephalon, the *primary optic vesicle.* Later it is transformed into the *secondary optic vesicle* (Fig. 546). Each optic cup remains connected with the brain by a stalk, the future optic nerve. The opening on the anterior pole of the secondary optic vesicle corresponds to the pupil of the iris in the adult eye.

In the adult this tunic consists of an outer pigmented epithelial layer (Figs. 534, 535, layer *1*) and an inner sheet, the retina proper (same figures, layers *2 to 10*). The latter does not elaborate pigment, except in the "macular" region, gradually increases in thickness, acquires a highly complex structure, and contains elements similar to those of the brain tissue; it may, therefore, be considered as a specially differentiated part of the brain. As the original cavity of the primary optic vesicle disappears, the two layers become adherent to each other.

The subdivision of the retina into three parts, the optic, the ciliary and the iridial portion, has been already mentioned. The *optical or functioning portion of the retina,* or the retina in the narrow sense, is the larger posterior portion of this membrane that lines the inner surface of the chorioid and extends from the papilla of the optic nerve in the fundus of the eye (Fig. 524) to the serrated margin anteriorly (Figs. 524, 528, *OS*). At the papilla, where the retina continues into the tissue of the nerve, and at the serrated

tinuous, radially fibrillated membrane which is called the posterior stromal sheet or lamella (Fig. 530, *FL, FQ*). In meridional sections it appears as a *distinct*, longitudinally striated layer between the vessel layer and the pigment layer (Fig. 530, *FL*). The undifferentiated parts of the cell

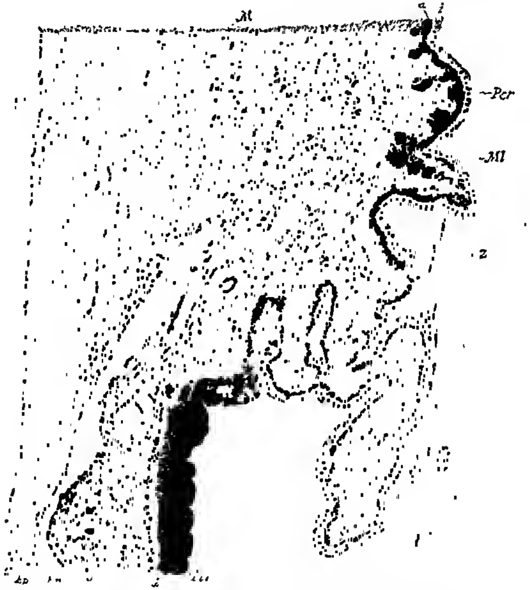

Fig. 532. Meridional section of the angle of the iris from an enucleated eyeball: *C*, Innermost layers of the corneal substantia propria; *Ca*, major arterial ring of the iris; *En*, endothelium; *Ep*, membrane of Descemet; *G*, dilator of the pupil; *J*, stroma of the iris; *Lp*, pectinate ligament of the iris; *M*, ciliary muscle, *Mi*, circular fibers of the ciliary muscle; *Pc*, ciliary process; *Pcr*, ciliary portion of the retina; *Pir*, iridial portion of the retina; *S*, canal of Schlemm; *Sk*, innermost layers of the sclera; *Z*, fibers of the ciliary zonule *a*, pigment epithelium of the ciliary portion of the retina; *i*, inner epithelial layer of the ciliary portion of the retina. 90 ×. After Schaffer.

bodies keep the elongated nucleus, accumulate pigment, are pushed backward and form a layer of pigmented spindle cells (Fig. 530, *ZK*). The latter are only indistinctly set off from the posterior stromal lamella (*FL, FQ*), but are sharply separated from the pigment epithelium (*P*).

Numerous muscular connections can be found between the sphincter and the dilator The innervation of both muscles, however, is quite different although both are supplied by visceral nerve fibers. The postganglionic neurons for the dilator are located in the superior *cervical* ganglion and are sympathetic. Their axons pass to the gasserian ganglion, thence into the *ophthalmic* branch of the latter and finally reach the dilatator through the long ciliary nerves. The postganglionic neurons for the sphincter *lie in* the ciliary ganglion and their axons reach the sphincter with the short ciliary nerves which also innervate the ciliary muscles; these are parasympathetic (p. 203). When the eye accommodates for a near object by the contraction of the ciliary

distinguished (Fig. 535). The inner three (*I-III*) compose the *central area* distinguished by the great number of ganglion where they are accumulated in greatest numbers. The regions outside the central area (*IV–VI*) including the ora serrata

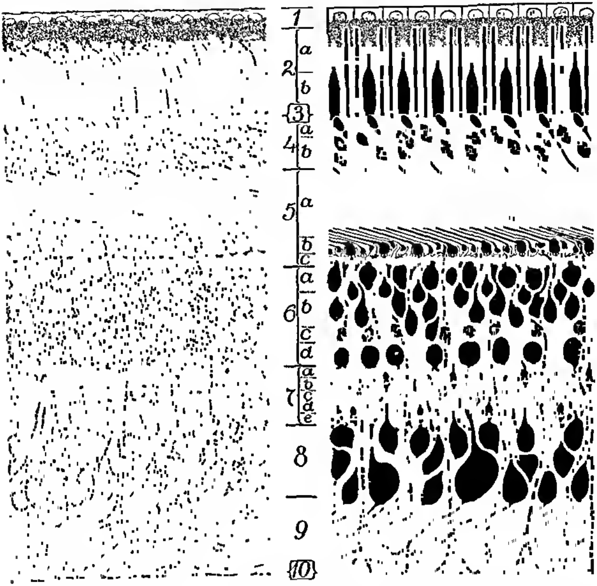

Fig. 534 Layers and sublayers of the adult human retina (region III). Left figure represents a cross section stained routinely, about 400 ×. The right figure is a schematic reconstruction from sections stained with Golgi's method (Figs 537, 539). *1*, Pigment epithelium; *2*, bacillary layer (thinner rods, thicker cones) composed of an outer zone *2-a* and an inner zone *2-b*, *3*, outer limiting membrane; *4*, outer nuclear layer with an outer, *4-a*, and an inner zone, *4 b*; *5*, outer plexiform layer with an outer zone *5-a* (Henle's outer fiber layer), a middle zone *5-b* (rod spherules and cone pedicles) and an inner zone *5-c*; *6*, inner nuclear layer with four zones, *6-a*, *6-b*, *6-c*, *6-d*; *7*, inner plexiform layer with five zones, *7-a*, *7-b*, *7-c*, *7-d*, *7-e*; *8*, layer of ganglion cells, *9*, layer of optic nerve fibers; *10*, inner limiting membrane.

cells in the eighth layer, and by the general refinement and the even distribution of the structural elements, especially of the rods and cones (*2*). The most delicate elements are in the fovea (region *I*) (*VII*), constitute the *extra-areal periphery*. Here the elements are larger but less in number, less differentiated and less evenly distributed. (Note that Roman numerals refer in text and legends to regions

margin, the retina is firmly connected with the chorioid.

At a distance of about 3.5 mm. from the border of the optic papilla the inner surface of the retina appears excavated. This shallow, round depression is called the *central fovea* (Figs. 524, 533, *f*; 535, *I*, *A*; 538).

When detached from the pigment epithelium the fresh retina is almost perfectly transparent. It has a distinctly red color which is due to the presence in the rods of a peculiar substance, the visual purple or rhodopsin (p. 618). Light rapidly bleaches the visual purple; in darkness the color gradually reappears.

The fovea, and its immediate vicinity, contains yellow pigment and is called the *yellow spot* (macula lutea).

Large blood vessels circle above and below the central fovea whereas only fine arteries and veins, and capillaries, are present in it (Fig. 538). In the very center of the fovea, in a territory measuring 0.5 mm. across, even the capillaries are absent, which greatly increases its transparency.

The position of the fovea on the inner surface of the fundus of the eyeball corresponds approximately to the visual axis (p. 602). Of the image of an external object which is formed on the retina, only the portion which falls upon the fovea is seen sharply. For distinct vision, therefore, the eyes are moved so as to bring the object of special attention into this central part of the visual field.

In the area of the optic papilla the elements necessary for the reception of the light stimuli are absent (Fig. 533). This is the "blind spot" of the visual field.

Layers of Retina. In a histological section vertical to the surface of the retina and stained with a nuclear dye (exclusive of the fovea, the papilla, and the serrated margin) ten parallel *layers* can be distinguished from outside inward (Fig. 534): (1) the pigment epithelium; (2) the layer of rods and cones (bacillary layer); (3) the outer limiting membrane; (4) the outer nuclear layer; (5) the outer plexiform layer; (6) the inner nuclear layer; (7) the inner plexiform layer; (8) the layer of ganglion cells; (9) the layer of optic nerve fibers, and (10) the inner limiting membrane (the numbers 1 to 10 correspond with those in Figs. 535–537, 539).

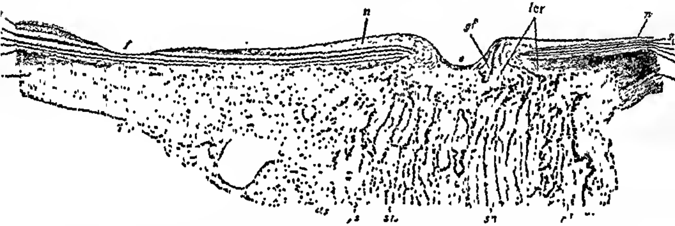

Fig. 533. Place of entrance of the optic nerve and the central fovea of an enucleated human eye in horizontal meridional section: *ak*, Outer nuclear layer; *ch*, chorioid; *d*, sclera; *ds*, dural sheath of optic nerve; *e*, physiologic excavation; *f*, central fovea; *g*, layer of ganglion cells; *gf*, blood vessels; *ik*, inner nuclear layer; *lcr*, lamina cribrosa; *n*, layer of nerve fibers; *p*, pigment layer of retina; *ps*, pial sheath of the optic nerve; *sn*, bundles of fibers of the optic nerve. 17 ×. After Schaffer.

Regions of the Retina. The distribution of the cellular and fibrous elements varies considerably in detail from the center of the retina in the fovea to its anterior limit at the serrated margin. Thus seven insular or circular *regions* can be

This is supposed to prevent the diffusion of light from one rod or cone to another. In an eye protected from light the pigment leaves the fringes and is massed in the cell body.

Visual Cells. These elements are the receptors of the light stimuli or photoreceptors (*2* and *4* in Figs. 534, 535, 536, 539). From their position it is clear that the light rays, before reaching them, must first penetrate the greater part of the thickness of the retina. The specifically differentiated outer portions of the visual cells are radially arranged and are believed to be the parts sensitive to light.

There are two kinds of visual cells: (*a*) the rod cells and (*b*) the cone cells.

Rod Cells. The rod cell (*a*) is a slender, filamentous element arranged with its outer portion vertical to the surface of the retina (Fig. 536, *a;* see also Figs. 534, 539). This causes the regular, radiated striation of the bacillary layer (2) in cross sections.

The outer or scleral part of the rod cell, the *rod* proper, is situated between the pigment epithelium (layer 1), its outward or scleral one third or more being embedded between the pigmented fringes, and the outer limiting membrane (layer 3). The inner or vitreal end of the rod proper extends for a short distance through the outer limiting membrane into the fourth layer. Each rod consists of an outer and inner segment The latter is a trifle stouter and longer than the first. The outer segment is a smooth cylinder of uniform thickness with a rounded outer end Its substance has a peculiar brilliancy, is homogeneous in life, and is positively birefringent

At the junction with the outer the inner rod segment contains a darkly-staining "fiber apparatus" and a diplosome In fishes and in birds the inner rod segment is contractile During illumination it lengthens and thus pushes the photosensitive outer segment toward the sclera deeper into the pigment epithelium In dim light the inner rod segment contracts, moving the outer segment closer to the outer limiting membrane, thus exposing it to more light This contractility of the inner rod segment is little known in *primates*.

The rods are fairly uniform in appearance. Their dimensions vary somewhat from region to region Their thickness in the central area (regions I–III) is 1 to 1.5 μ, gradually increasing to 2.5 or 3 μ near the ora (region VII). Inversely their length decreases in the same direction from approximately 60 μ in the fovea to 40 μ in the far periphery.

The rest of the rod cell is made up of the *rod fiber* and the *rod body* (Figs. 534, 536, 539).

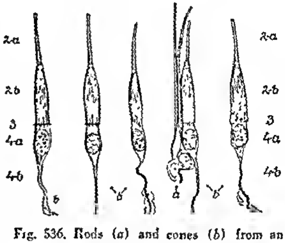

Fig. 536. Rods (*a*) and cones (*b*) from an osmic acid fixed, unstained, teased preparation of the retina of a rhesus monkey (preparation of G. W. Bartelmez). Designation of layers as in Fig. 534. Outer rod and cone segments in zone *2-a*, inner segments in *2-b*, rod bodies with their nuclei in zone *4-b*, cone bodies with nuclei in *4-a; 3,* outer limiting membrane, parts of which with fiber baskets still adhering to the bases of the inner cone segments; fiber apparatus visible in the outer (upper) portion of the inner cone segments. Intermediate zone incorrectly called "vacuole," between two cone segments visible as a bright belt. Some of the outer segments slightly bent or deformed. Camera lucida.

It extends from the lower attenuated end of the rod proper through the fourth layer (*4*) to the middle zone of the fifth layer (*5-b*) where it terminates with a tiny, round swelling, the *rod spherule*, smaller than the analogous cone pedicle. The rod fiber is a delicate protoplasmic thread of smooth appearance and uniform thickness which does not exceed 1 μ; its length varies considerably. The course of the rod fibers is vertical in the extra-areal regions, while in the central area it assumes a slanting to horizontal position (cf. zone *5-a* in Figs. 534, 535).

Along the rod fiber is the *rod body* containing a *nucleus*, smaller and more intensively stained than the cone nucleus, and surrounded by scant *protoplasm*. The outer fiber together with the rod proper is the homologue of the receptive dendritic expansions, the inner rod fiber of the emissive axis cylinder, of a neuron (p. 181). In

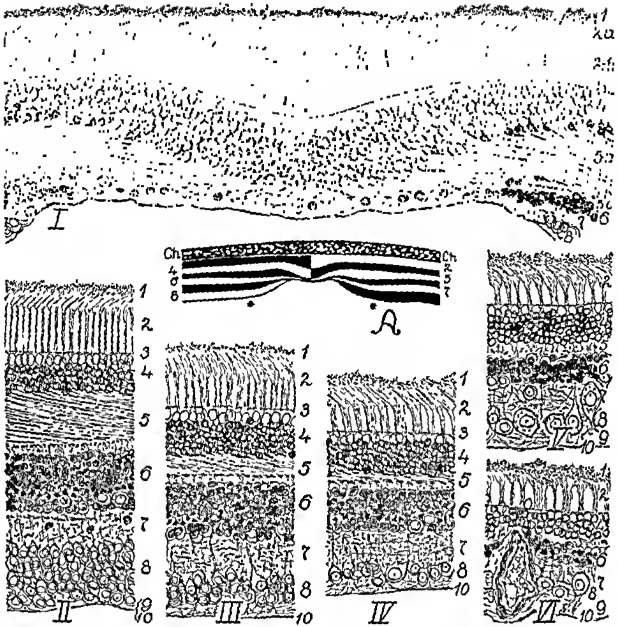

Fig. 535. Samples from various regions of the retina of a rhesus monkey. *A*, Diagram of the fovea showing alternating layers differently presented in the right and left halves, with the dividing line in the foveal center; asterisks indicate the margins of the central or inner fovea (region I); the outer fovea is indicated by the dip downward of the line separating layer 2 from layer 4; Ch, chorioid membrane, other designations as in preceding figure. *I*, Floor of the inner or central fovea, and the outer fovea (center of region *I*); *II*, perifoveal region; *III*, periphery of the central areas; (*I*, *II*, and *III* together are the *central area*); *IV*, near periphery; *V*, middle periphery; *VI*, far periphery (these with region *VII*, the extreme periphery near the ora serrata, represent *extra-areal* periphery). *I* reproduced at a somewhat higher magnification than the others (*II–VI*).

of the retina, while the Arabic refer to the layers.)

Pigment Epithelium. This layer consists of one row of low prismatic cells (layer 1 in Figs. 534, 535, 539). When seen from the surface, they usually appear as fairly regular hexagons of an average diameter of 16 μ. In cross section the same cells appear as rectangles 8 μ high. In the region of the fovea the cells are *narrower* (10 μ) and taller (10 to 14 μ). The inner surface of the cells sends out thin, fringelike, protoplasmic processes (10 to 40 to a cell) filled with pigment (fuscin), which surround the rods and cones and separate them from one another. The outer part of the protoplasm adjacent to the chorioid is free of pigment and contains an oval nucleus.

With changes of illumination these pigment inclusions change their position. In an eye which has been exposed to light the rod-shaped pigment granules move into the processes, thus providing each rod and cone with a pigment sheath.

This is supposed to prevent the diffusion of light from one rod or cone to another. In an eye protected from light the pigment leaves the fringes and is massed in the cell body.

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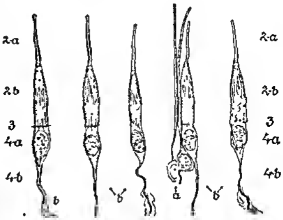

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the central area the inner rod and cone fibers and the corresponding portions of Müller's fibers (p. 622) which envelop the first two form a thick fiber layer, the outer fiber layer of Henle (zone *5-a* in Figs. 534, 535, *I, II, III*). The rod nuclei, dark-shaded in left-hand drawing in Fig. 534 and in Fig. 535, represent the majority of the nuclei of the fourth layer (*4*) in all regions except in the fovea where they are few, and in its center where they are absent (*I* in Fig. 535).

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The number of rods in the human retina, according to Krause, is 130 millions.

Cone Cells. These neurons (*b*) are made up of essentially the same parts as the rod cells but differ in detail. On the whole, they are bulkier. The outward portion instead of being a slender rod is a thick, flask-shaped structure.

The part situated outward to the outer limiting membrane (*3*) is the *cone* or the cone proper (Figs. 534, 535, 536, 539). It, too, is divided into two parts. The highly refractive and fragile outer segment is a long, slender cone with straight, smooth outlines (Fig. 536). It rests with a broader base upon the stout inner segment, and it tapers toward its blunt outward tip (the swollen tip often seen is an autolytic artefact). It is somewhat more resistant than the outer rod *segment*. The *inner cone segment varies* in shape and size from place to place. In the central region and close to it, its shape is that of a cylinder, gradually assuming the likeness of a bottle or a barrel in the periphery (Fig. 535, *I–VI*). As the rod, it contains a "*fiber apparatus*" and a diplosome (first clearly visible in Fig. 536). Also, it is much more resistant to physical and chemical agents than the outer segment. There is no visual purple in the cones; it is possible that they contain a similar substance which is colorless.

The dimensions and the shape of the cones vary considerably in different regions of the retina. In the central fovea the long and slender cones measure 75 μ *or more in length and from* 1 to 1.5 μ in thickness (at the tip of the outer and at the base of the inner segment, respectively, p. 622). Their length gradually decreases to 40 or 45 μ in the extra-areal periphery. Here it is in general from one sixth to one fourth less than the length of the rods. Their thickness at the base of the inner segment at the same time increases from three to five times the thickness of the inner rod segment of the same locality. The usual relative length of the outer and the inner cone segment is as three to four; this, however, varies. In the fovea the two are approximately of the same length or the outer segment exceeds some*what the inner one.* As in the rods, the lower end of the inner cone segment slips through an opening in the outer limiting membrane (*3*), and protrudes slightly into the fourth layer. In the cone the opening is larger than in the rod. Where the cone is fitted into the membrane it is, as is the rod, somewhat indented.

In certain vertebrates (teleostean fishes, amphibians) the inner cone segment, too, is contractile. *It shortens in bright light* and stretches in dim light or darkness. It is not certain whether human cones possess the same property. The displacement of the cones, where present is, accordingly, opposite in direction to that of the rods.

Inward to the outer *limiting* membrane *the* inner cone segment merges with its *body* containing a *nucleus*, which is larger and paler staining than the rod nucleus (Fig. 536). The bodies and nuclei of the cones, as distinct from those of the rods, are placed in a single row (*4-a*) immediately beneath the outer limiting membrane (Figs. 534, 535, *II–VI*, 539). The exceptions are the cones in the outer fovea whose nuclei, accumulated in several rows, are at a short distance from the membrane (Fig. 535, *I*). Only here the cones possess an *outer fiber*. From the body of all cones a stout, smooth *inner fiber* descends to the middle zone of the outer plexiform layer (*5-b*) *where it terminates* with a thick triangular or club-shaped swelling, the *cone pedicle* (right-hand figure in Fig. 534, and Fig. 539). Up to a dozen short, barblike filaments emanate from the base of each pedicle, except in the fovea where *there are usually none.* These filamentous outgrowths spread horizontally in zone *5-c*. The length and course of the inner cone fibers vary considerably depending on the region, the longest (600 μ) and almost horizontally placed being

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The total number of cones in the human retina is estimated at from 6,000,000 to 7,000,000 (Østerberg). The ratio of the nerve fibers of the optic nerve (438,000) to the number of cones of one eye is as 1:6 or 1:7. The relative number and distribution of the rods and cones in different vertebrates, depending on the mode of life, present great variations. In diurnal birds the cones are more numerous than the rods. In most diurnal reptiles there are only cones and no rods. On the contrary, in many nocturnal vertebrates only rods are present; still in others a few rudimentary cones can be found among numerous rods (Kolmer). On similar comparative data M. Schultze (1866) assumed a difference in the function of the two kinds of photoreceptors (duplicity theory).

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Horizontal Cells. These cells (*c*) are typical neurons whose bodies form the uppermost one or two rows of the inner nuclear layer (zone *6-a* in Figs. 534, 539).

From the upper end of the body arise short dendritic twigs producing several tufts spreading in the lower zone of the outer plexiform layer (*5-c*), where each tuft comes in contact with the vitreal face of one cone pedicle. The axis cylinder arises from the neck or from one of the short main dendrites and takes a horizontal course chiefly in zone *5-c* (hence the name of this neuron). After a considerable course it divides into a great number of branchlets, thus producing a teledendron. Its terminal twigs come in contact both with the rod spherules and cone pedicles. The horizontal cells (*c*), accordingly, receive impulses from a group of cone cells (*b*) of one locality and transmit these to another group of both rod cells (*a*) and cone cells (*b*) of another locality.

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Depending on the appearance of the expansions and on the mode of synapsing, two groups of bipolars can be distinguished: centripetal bipolars which transmit impulses from rods and cones to ganglion cells (*d*, *e*, *f*, *h*) and centrifugal bipolars which transmit impulses in the opposite direction (*i*). The bipolar cells apparently play an essential rôle in distributing and rearranging the impulses received from the rods and cones before

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transmitting them to the third category of retinal neurons, the ganglion cells.

Ganglion Cells. These cells (*m, n, o, p, s* in Fig. 539, and layer *8* in Fig. 534) represent the third link, the last in the retina, of the chain of neurons that form the afferent visual pathway. These cells are larger than those of the two nuclear layers, and closely resemble the neurons of the brain. The larger cells are characteristic of the periphery, the smaller of the central area, particularly of the fovea, where they are most numerous.

The bodies are placed in the ganglion layer (*8*). A few are displaced into the lowermost zone of the inner nuclear layer (*6-d*). Their dendrites spread in the inner plexiform layer (*7*). Nissl's chromophil substance is present in all.

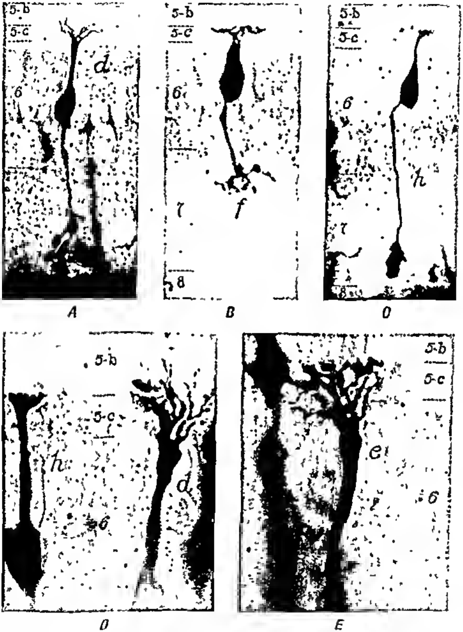

Fig. 537. Photomicrographs of different varieties of centripetal bipolar cell; variety *d* (in A and in D), variety *e* (in E), variety *f* (in B), variety *h* (in C and in D). Method of Golgi. Rhesus monkey. A, B, C at low, D and E at high magnification. Designation of layers as in Fig. 534.

From the body or the chief dendritic trunk of each ganglion cell arises one axis cylinder that leaves the retina and becomes an optic nerve fiber which terminates with a teledendron in the subcortical visual centers of the brain.

Arrangement and Course of the

Optic Nerve Fibers in the Primate Retina. Because of the presence of the central fovea, the optic nerve fibers have a special course (Fig. 538). While in general all optic nerve fibers converge radially toward the optic papilla, mostly taking the shortest course, in the temporal half only a part of the fibers originating in the nasal portion of the central fovea pass more or less directly to the temporal edge of the papilla. (These are usually labeled the papillo-macular bundle.) The fibers arising from both temporal quadrants of the retina bend above and below the horizontal meridian and thus reach the optic papilla. As a result, all fibers originating in the upper temporal quadrant of the retina circle above the central area or

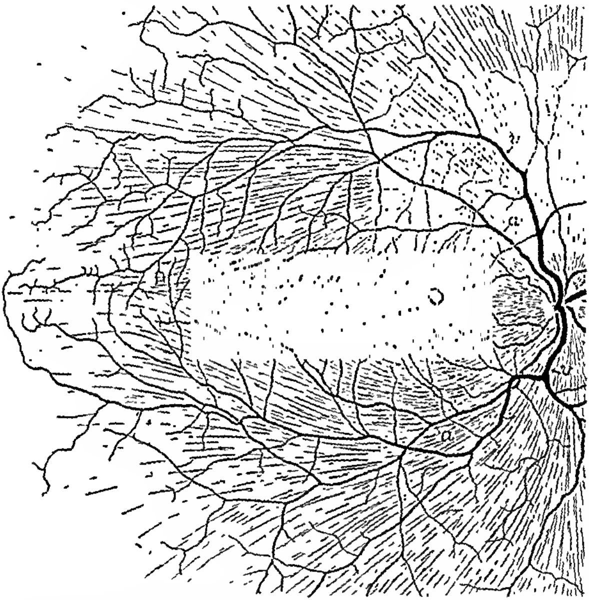

Fig. 538. Retina of the right eye of an adult rhesus monkey as seen in a total preparation. Intravital staining with methylene blue (Ehrlich). The elliptic papilla of the optic nerve is near the right side of the figure. Fine lines radiating from the papilla in all directions represent bundles of optic nerve fibers. The stippled circular area almost free from fibers is the central fovea. Within it a small white circle is the floor of the fovea; the dark ring surrounding it is the foveal slope. Note the direction of some of the foveal fibers straight to the fovea, of others more or less circling to it or around it, encircling the foveal region from above and below, and forming on the temporal side to it (left) the horizontally placed "raphé" or retinal seam. *a*, Arteries; *v*, veins. Camera lucida. 8 ×.

fovea on their way to the optic papilla, while those originating in the lower temporal quadrant circle below the central area, following fairly closely the larger retinal vessels (*a*, *v*, in Fig. 538).

A horizontal line connecting the fovea with the temporal circumference of the retina is the line of cleavage from where the optic nerve fibers proceed either upward or downward. This "raphé" thus separates the optic nerve fibers of the upper from those of the lower temporal quadrant.

This principle of separation of the upper from the lower quadrantic fibers is preserved along the central visual pathway as far as the cortical visual center.

Another peculiarity of the afferent visual pathway in primates is the division of each retina into two halves along a vertical line, the vertical meridian, passing through the center of the fovea. The fibers originating in the nasal half cross in the chiasma of the optic nerves and pass to the optic tract of the opposite side, those originating in the temporal half enter into the tract of the same side. Each optic tract is, *therefore*, composed of fibers from the temporal half of the same side and from the nasal half of the retina of the opposite eye. This arrangement likewise remains in the visual radiation in the occipital lobes of the brain. It accounts for the blindness in the opposite halves of the two fields of view (homonymous hemianopia) when the optic tract or the visual radiation of one side is interrupted. (The crossed blindness is due to the crossing of the rays in the eyes.)

Supporting or Neuroglial Elements of the Retina. The retina, a modified part of the brain vesicle, contains supporting elements of neuroglial character. The most important are the radial fibers of Muller (*u* in Fig. 539). These are present everywhere in the central area including the fovea, as well as in the periphery.

Their oval nuclei lie in the middle zone of the inner nuclear layer (*6-c*, right-hand drawing in Fig. 534) The cell body is a slender fiber or pillar which extends radially from the outer (*3*) to the inner limiting membrane (*10*). Their inner ends expand conically and together form or appear as the *inner limiting membrane* (*10* in Figs. 534, 535, 539). In the two plexiform layers the radial fibers give off many branches, which form a dense neuroglial network in whose meshes the ramifications of the various neurons described above are lodged.

In the ganglion layer and in the inner and outer nuclear layers, the cell bodies of the radial fibers are beset with numerous socket-like excavations enveloping bodies of the ganglion cells, bipolars, horizontals, and of the cone and rod cells. The bodies of the nervous elements appear to be completely enveloped in very thin husks of supporting structures, and are in such a way insulated; the rod and cone fibers are likewise encased in thin tubelike sheaths produced by Müller's fibers.

At the limit between the outer nuclear layer and the layer of the rods and cones the radial fibers fuse in the tangential plane and form the *outer limiting membrane* (*3* in Figs. 534–536, 539). This is pierced by numerous openings through which the rods and cones are connected with their inner parts, their bodies.

Central Area and Fovea ("Macula"). In the region not far lateral to the optic papilla the structure of the retina presents important alterations which are adaptations of this locality to its function as the place of distinct vision. The chief characteristics of this region is the agglomeration of cones and other nervous elements in numbers greater than outside it and their structural refinement and synaptic perfection. This is the *central area*. In addition, in the center of this area, the layers inward to the zone *5-a* are displaced laterally, producing a shallow depression on the vitreal surface of the retina called *central fovea*. This permits an almost free passage of the rays of light to the layer of photoreceptors (*2*). It is here where the visual axis touches the retina.

The central fovea has the shape of a round or slightly elliptical, shallow bowl with its concavity toward the vitreous (Fig. 535, *I* and *A*). It is in the center of the central area at a distance of 2 or 2.5 mm., or somewhat more, on the temporal side of the papilla In its center a *floor* or *fundus* can be distinguished, with the *slopes* and a *margin of the fovea*. The width from edge to edge of the entire foveal depression measures 1.5 mm.

In the fundus of the central fovea, the cones

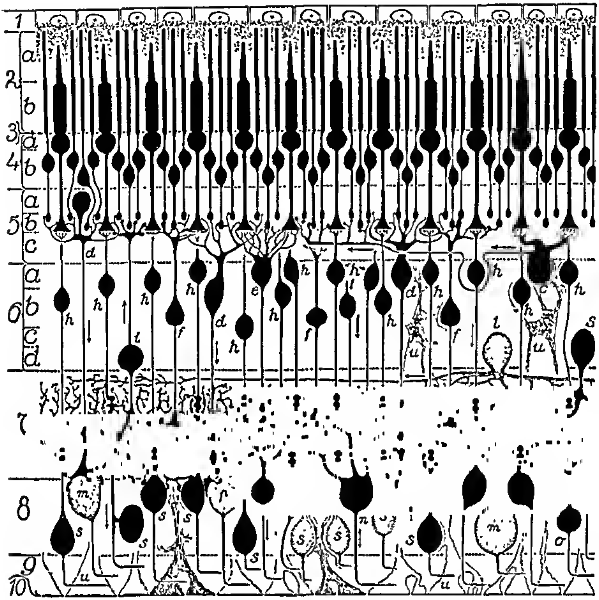

Fig. 539. Diagram representing the structures of the primate retina composed from numerous Golgi-stained preparations of man, chimpanzee and macaque. The designation of layers and zones on the left side as in Fig. 531. In the upper part the slender structures are the rod cells (*a*), the thicker ones the cone cells (*b*); *c*, horizontal cell; *d*, *e*, *f*, *h*, centripetal bipolar cells; *i*, centrifugal bipolar cell; *l*, inner horizontal or association cell; *m*, *n*, *o*, *p*, *s*, ganglion cells; *u*, parts of the radial fibers of Müller, with their nuclei in *6*, and their lower or inner ends forming the inner limiting membrane (*10*). Note the various synaptic relations between different neurons, reciprocal overlapping of expansions or its absence, the probable direction of the nervous impulses indicated by arrows, and other details. The indicated termination of the l-axon is not completely proved. In this figure the rods and the cones are not designated by letters *a* and *b* as in Fig. 536.

are most numerous and are thinner and longer than elsewhere in the retina. This formation is the *outer fovea*.

The *number of cones* in the outer fovea is from 20,000 to 25,000. This region, which is almost the same as the foveola or the unobstructed floor of the inner fovea, measuring 400 μ or somewhat more across, very likely corresponds to the portion of the field of view where vision is most discriminating. The *rod-free area* where only cones are present, measures from 500 to 550 μ across. Here the number of cones is up to 30,000. The foveal cones are morphologically as distinct from the rods, in spite of their reduced dimensions and superficial similarity, as are the voluminous cones outside the fovea.

Capillaries are present in the ventral layers of the foveal slopes to the very edge of the foveal

floor or foveola, or 275 μ from the very center. The *avascular central territory* is almost as large as the rodless area (500–550 μ).

Function of the Eye. Synapses and Function of the Retina. The human eye and that of all other vertebrates is essentially a dark chamber (camera obscura) provided with dioptric media. Two of these, the cornea with the aqueous humor and the crystalline lens, placed immediately in front and behind the pupillary opening, respectively, are optically active, the lens, in addition, being adjustable. The back part of the inner surface of this dark chamber is lined by a photosensitive screen, the retina. The countless rays of light emanating from each point of a luminous or illuminated object radiate in all directions in straight lines. A portion of the rays from a certain point caught by the cornea is first refracted by it in such a way as to make it converge upon the lens which it reaches through the pupil. In the lens again the rays are further refracted and assembled into a cone that converges upon one point, the focus, which is in the photosensitive bacillary layer of the retina (2 in our figures) and opposite its outside source. The sum total of such separate foci, that symmetrically almost exactly correspond with the points on the surface of the object seen, constitutes the retinal image of this object. In relation to the object the retinal image is inverted (because of the crossing of the rays in the pupil's aperature), real (since the foci are actually on the retina and not behind or in front of it) and very much reduced in size.

Stripped of many important details, the complex story of the interneuronic relationships in the retina may be told as follows:

In the photosensitive bacillary layer, in the rods and the cones (2, probably 2-a, Fig. 539), the light initiates or elicits a specific nervous process which in turn produces a train or trains of nervous impulses which are forwarded along the nervous pathways to the brain. Subjectively this is interpreted as light, colors, shapes, sizes, position, movement and distance in space of the objects seen.

The *synaptic mechanism* of the retina is composed of the following systems of neurons (Fig. 539). The rod cells (*a*) transmit impulses to two or three varieties of bipolars (*d*, *e-f*) and through these to all varieties of the ganglion cells (*m*, *n*, *o*, *p*, *s*). The cones (*b*), on the contrary, discharge impulses to all three or four bipolar varieties (*d*, *e-f*, *h*), and through these to all varieties of ganglions (*m*, *n*, *o*, *p*, *s*); the cones stimulate also the horizontal cells (*c*) and thus may influence distant rods (*a*) and cones (*b*).

The rods, it is believed, are responsive to weak light stimuli in general, thus being adapted for seeing in dim light. They, however, do not selectively respond to lights of different wave lengths associated with the sensations of "colors." Conversely, the "color" sensations are initiated through the stimulation of cones otherwise less responsive to weak stimuli of the diffuse "colorless" light. This makes the cones especially suitable for daylight or photopic vision.

At the third neuronal level, in the ganglion cells (*m*, *n*, *o*, *p*, *s*), since all three bipolar varieties (*d*, *e-f*, *h*) are synaptically connected with each of the several ganglion varieties, the rod and cone impulses apparently merge with one another. The further fate of the impulses in the brain itself is unknown.

The synaptic relations suggest that the *cones react in a way that is territorially more restricted than the rods.* In and near the central fovea, each cone (*b*) is linked to one h-bipolar which in turn is related to a single s-ganglion cell. This set of neurons (*b-h-s*) may well form a spatially differentiated receptor-transmitter apparatus (Fig. 193, *B*). This implies that the visual system is made up of a great number of minute and almost equivalent ana-

tomical and functional units, each of which responds independently to a minute photic stimulus. This may be the structural basis for *visual space perception* or *visual acuity.*

In the system of rods a slight degree of localization must also be accepted. Yet the rods, being connected in groups to bipolars, always respond in groups no matter how restricted the photic stimulus may be. Thus even the smallest receptive rod territory is larger than the cone territory of the same region. The possible effect resulting from such grouplike connections seems to be the *reinforcement of the intensity of excitation* generated in the rods (Fig. 193, *D*).

In the central area the size of the receptor-conductor units in the system of cones corresponds roughly with that of the individual cones. This agrees with the difference in retinal acuity in different localities, the acuity being at its peak in the very center of the field of view (corresponding with the foveal center) and at first rapidly, then more slowly decreasing toward the periphery of the field of view (carresponding with the anterior limit of the retina at the ora serrata).

In the primate retina at least 15 distinct varieties of neurons are present; these form at least 38 kinds of synapses with one another. In the retina, in addition to photoreception, many other processes usually associated with the central nervous system—as selection, facilitation, inhibition, summation of excitations, etc.—take place. The retina is thus essentially a *receptor-integrator organ.* (For further details see Polyak, The Retina and The Symposium on "Visual Mechanisms", edited by Kluver.) The metabolism of the retina is reviewed by Krause and Sibley.

THE REFRACTIVE MEDIA OF THE EYE

The cornea and the two chambers of the eye have been described earlier.

Lens. The lens is a transparent, round, elastic body biconvex in shape, placed behind the pupil. Its outer form varies somewhat in different individuals and also with age. Its form likewise changes during the process of accommodation. Its diameter is from 7 mm. in a newborn up to 10 mm. in adult. Its thickness is approximately 3.7 to 4 mm., increasing during accommodation to 4.5 mm. and more. The posterior surface or pole is more convex than the anterior, the respective radii of curvature, according to Helmholtz, being 6.9 and 10 mm. The index of refraction is 1.36 in the peripheral layers and 1.42 in the inner zone or nucleus. The weight of the lens is 0.2 gm., its color slightly yellow.

The surface of the lens is covered with a homogeneous, highly refractive *capsule* (Fig. 540, *K*), a thick cuticular membrane. The inner surface of the anterior sheet of the capsule that covers the anterior face is lined with a single layer of flattened hexagonal cells, the epithelium of the lens (Fig. 540, *E*). Toward the equator these approach the columnar form and become arranged in meridional rows. Becoming progressively elongated the epithelial cells at the equator are transformed into *lens fibers* that constitute the tissue of the lens. This transition causes, in meridional sections, a peculiar arrangement of the cells with their nuclei, the so-called *nuclear zone, nuclear arc* or *lens vortex* (Fig. 540, *KZ*). The inner (anterior) surface of the posterior sheet of the capsule that covers the posterior pole has no epithelium and directly covers the lens fibers. The epithelial cells are of prime importance for the normal metabolism of the lenticular tissue.

In the human lens the length of the fibers is from 7 to 10 mm. Each fiber is a six-sided prism, 8 to 12 μ wide and only 2 μ thick (Fig. 541). In the region of the nucleus the thickness may reach 5 μ. In younger fibers a firmer cortical and a semiliquid axial part can be distinguished. With advancing age the axial part, too, becomes increasingly solid (sclerosis). The young fibers

have smooth surfaces and join one another so that their narrow edges interdigitate. They are kept together by thin layers of a cementing substance which has the same index of refraction as the fibers themselves. This substance is considered by some to be a lubricant enabling slight movements of the fibers during accommodation. In the older fibers, in the dense, inner portion of the lens, the nucleus is absent and the outlines

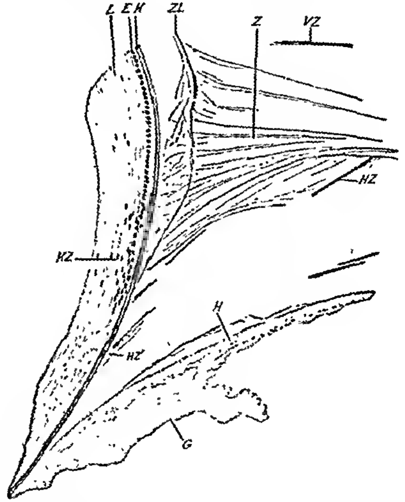

Fig. 540. From a meridional section of the anterior part of the eye of a man. Attachment of the fibers of the zonula ciliaris to the equator of the lens: *E*, Epithelium of the lens; *G*, vitreous *body*; *H*, membrana hyaloidea; *HZ*, posterior fibers of the zonule; *HZ'*, their connections with the capsule of the lens (*K*); *KZ*, nuclear zone of the lens; *L*, fibers of the lens substance in longitudinal section; *VZ*, anterior fibers of the zonule; *Z*, zonule; *ZL*, detached outer layer of the capsule of the lens. 110 ×. After Schaffer.

of the cross sections often become irregular and serrated. At the two poles of the lens where the fibers join their ends, they form a figure of a star with three or more rays (Fig. 528, *L*).

Ciliary Zonule. The lens is held in its position by a system of fibers—the *ciliary zonule*. The zonule fibers (Fig. 540, *Z*, *VZ*, *HZ*) are straight, homogeneous filaments varying in thickness (up to 22 μ or more) and having many branches. They seem to arise from the surface of the ciliary epithelium. Many of them, especially the thinnest ones, begin on the surface of the ciliary portion of the retina and, taking a meridional course, cause the fine striation of the surface of the ciliary ring. In the region of the ciliary crown they fuse into thicker fibers which occupy a more central position and there they continue to receive numerous, thin reinforcements from the surface of the ciliary processes. The number of the bundles is about 140.

As the bundles reach the anterior margin of the ciliary processes they leave the surface of

the ciliary body and, keeping their meridional course, radiate freely toward the equator of the lens. The larger among them, which constitute the majority, are straight and reach the capsule at the anterior periphery of the lens, in front of its equator. These are also called the *anterior sheet of the zonule*. The thinner fibers assume a slightly curved course, with the convexity forward, and are attached to the posterior surface of the lens. They form the *posterior zonular sheet*. Reaching the capsule of the lens, all zonular fibers break up into a multitude of fine brushlike fibers which fuse with the substance of the outermost layer of the capsule.

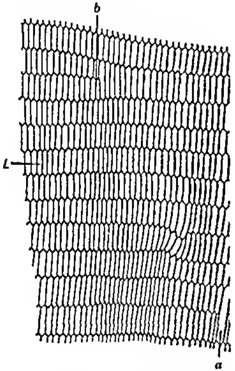

Fig 541. Part of a frontal section through the equator of the human lens. The cross sections of the fibers are arranged in radial rows or lamellae (*L*) : *a*, Fiber of double width; *b*, branching of a radial lamella. 500 ×. After Schaffer.

Where the vitreous is in touch with the posterior surface of the lens, it adheres to the peripheral zone of the lens capsule and forms the circular *hyaloideocapsular ligament* (Fig. 529, *LHK*).

The radii of curvature of the surfaces of the several dioptrical media of the normal (emmetropic) eye, especially of the lens, and their indices of refraction are such that light rays coming from a remote point, i.e., parallel rays, must give an inverted and real image of the object in the retina exactly in the layer of the photoreceptive elements, the cones and the rods (2 in Figs. 534, 539). If the object is approaching, the light rays become more and more divergent and the image moves backward. A change of the position of the object from infinite distance to about 5 meters causes a shifting of the image of about 60 μ backward. Since under these conditions the image still remains within the layer of the outer segments of the rods and cones, accommodation is not needed. For nearer distances accommodation is necessary.

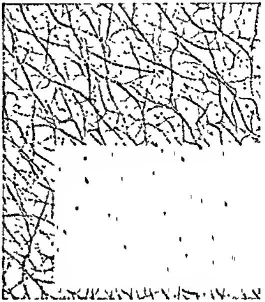

Fig. 512. Structure of the vitreous body. 500 ×. After Schaffer.

In a photographic camera the focusing of objects which are moved nearer to the lens is effected by moving the ground glass plate away from the lens. In the higher vertebrates and in man, the curvature of the lens is changed. The lens is an elastic body possessing an inner tension due to its peculiar structure. When the eye is at rest the lens is kept stretched in the plane vertical to the optical axis by the ciliary zonule. When the eye has to focus a near object, the ciliary muscle, especially its meridional fibers (tensor of the chorioid) contracts and pulls the chorioid with the ciliary body forward. This relieves the tension exerted by the zonule, the lens gets thicker and its surface, especially at the anterior pole, becomes more convex. In this way the refractive power of the lens increases, which in turn keeps the focus within the bacillary layer.

Vitreous Body. The vitreous body

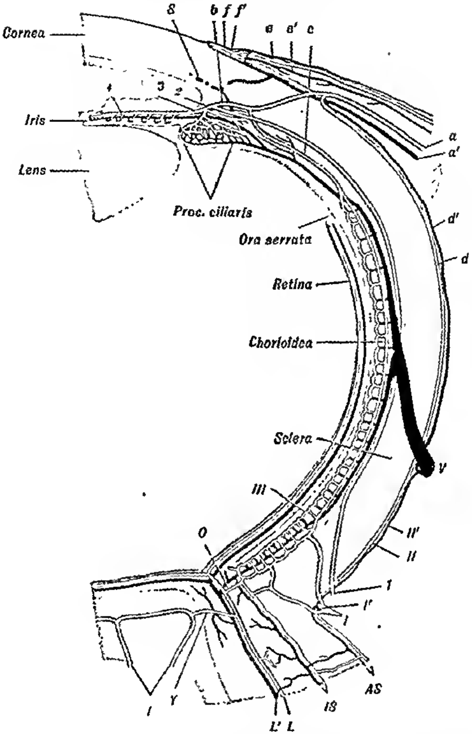

Fig. 543. Diagram of the distribution of the blood vessels in the eye. The arteries are double contoured, the veins are solid black. *L* and *L'*, Central retinal artery and vein; *Y*, anastomosis with the branches of the short posterior ciliary arteries; *O*, anastomosis with the vessels of the chorioid; *IS*, vessels of the inner and, *AS*, of the outer sheath of the optic nerve; *I* and *I'*, short posterior ciliary arteries and veins; *II* and *II'*, their arterial and venous episcleral branches; *III*, capillaries of the chorio-capillary lamina; *1*, posterior long ciliary artery; *2*, cross section of the major circle of the iris; *3*, its branches to the ciliary body; *4*, its branches for the iris; *a* and *a'*, anterior ciliary artery and vein; *b*, their connection with the major circle of the iris; *c*, their connection with the choriocapillary lamina; *d* and *d'*, their arterial and venous episcleral branches; *e* and *e'*, their arterial and venous branches to the scleral conjunctiva; *f* and *f'*, their arterial and venous branches to the margin of the cornea; *V*, vena vorticosa; *S*, cross section of the canal of Schlemm. Note: The anastomoses *O* and *Y* are usually capillaries or precapillaries; the central artery is functionally a terminal artery. After Leber and Stöhr, slightly modified.

fills the space (vitreal cavity) between the lens and the retina (Fig. 524). It adheres everywhere to the optical portion of the retina and the connection is especially firm at the serrated margin. Farther forward it gradually recedes from the surface of the ciliary portion of the retina.

The substance of the vitreous body in fresh condition has a gelatinous consistency, is colorless, structureless and of glasslike transparency. Its index of refraction is 1.334. In fixed sections it shows a spongelike network of extremely fine fibrils with its meshes filled with clear liquid (Fig. 542). Almost 99 per cent of the vitreous consists of water and its dissolved substances

From the papilla of the optic nerve to the posterior surface of the lens *the hyaloid canal* (Cloquet) extends through the mass of the vitreous body. It is a residue after the resorption of the embryonic hyaloid artery. It has a diameter of 1 mm. and is filled with aqueous liquid. It can be seen distinctly with the help of the ultramicroscope and in injected preparations. In the living, especially in young individuals, it is visible with the help of the corneal microscope and the slit lamp.

In the peripheral layers of the vitreous, free cells float in the liquid. They are probably lymphoid wandering cells of hematogenous origin.

Blood Vessels of the Eye. They arise from the ophthalmic artery and can be subdivided into two groups which are almost completely independent and anastomose with each other only in the region of the entrance of the optic nerve. The first group, *the retinal system*, represented by the central artery and vein (Fig. 513, *L'*, *L*), supplies a part of the optic nerve and the retina. The second, *the ciliary system*, is destined mainly for the uveal tunic (Fig. 543, *I'*, *I*).

Lymph Spaces of the Eye. True lymph capillaries and lymph vessels are present only in the scleral conjunctiva. In the eyeball they are absent.

Artificial injection into the eyeball has shown that liquid can find its way in different directions. Thus, a mass injected into the space between the chorioid and sclera penetrates along the walls of the vortex veins into the *space of Tenon*. The latter continues as the *supravaginal space* along the outer surface of the dural sheath of the optic nerve to the optic foramen. Again, it is possible to inject Tenon's space from the subarachnoid space of the brain. From *the anterior chamber* the injected liquid passes into *the posterior chamber*, and also into Schlemm's canal. All of these spaces cannot, however, be considered as belonging to the lymphatic system. The space of Tenon is more like a joint cavity and facilitates the movements of the eyeball.

The *aqueous humor* is believed to originate through secretion or transudation from the ciliary processes. From the posterior chamber it permeates the vitreous. In the forward direction it penetrates between the lens and the iris and through the pupil into the anterior chamber. The drainage of the aqueous humor from the anterior chamber is effected mainly through the spaces of Fontana and the canal of Schlemm. The normal intra-ocular pressure (28 mm. mercury), which causes the spherical form of the eyeball, is the resultant of the rates of transudation and of drainage of the aqueous humor. In pathological conditions (glaucoma) the intra-ocular pressure may increase considerably. On the other hand, it is possible that the equilibrium between the blood plasma and the aqueous humor is maintained through the action of molecular forces Drainage through the canal of Schlemm seems doubtful and it is possible that the liquid from the anterior chamber is resorbed by the crypts, by the blood vessels of the iris, and by the perivascular spaces of the episcleral and vortex veins.

Nerves of the Eye. These are the optic nerve, supplying the retina, and the ciliary nerves. The latter supply the eyeball with motor, sensory, and sympathetic fibers.

The *optic nerve* develops as an evagination of the prosencephalon, the optic vesicle (Fig. 516, *St*). It is not a peripheral nerve like the other cranial nerves, but a tract of the central nervous system, as found in any part of the white substance (therefore "fasciculus opticus" in the new terminology). It consists of about 1200 bundles of myelinated fibers without neurilemma. The nerve fibers are kept together by the same kind of neuroglia as in the white substance of the central nervous system. On the surface of each bundle the glia forms a thin limiting membrane which separates the nervous elements from the connective tissue. A similar layer is also found at the periphery of the optic nerve. The bundles are separated from one another by a system of thin, branching, connective tissue lamellae which are continuations of the pia mater on the surface of the nerve and carry the nutrient blood vessels.

The meninges and the intermeningeal spaces of the brain continue upon the optic nerve. The outer sheath of the nerve is formed by the dura,

which continues toward the eyeball and fuses with the sclera. The pia mater continues upon the nerve and forms a connective tissue layer which is closely adherent to its surface. Its tissue also fuses with the sclera at the entrance of the optic nerve. This pial layer sends the con- openings in the lamina cribrosa, the fibers get their myelin sheaths. The central artery and the central vein reach the eyeball through the optic nerve; they penetrate the nerve on the lower side at a distance from the eyeball varying from 5 to 20 mm., usually 6 to 8 mm.

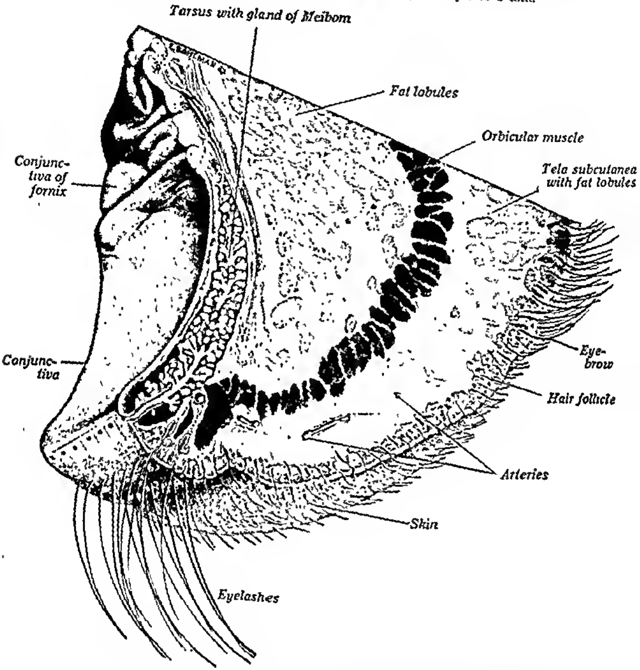

Fig. 544. Camera lucida drawing of a slice of the upper eyelid of a newborn infant. Stained with hematoxylin. 12 ×. Drawn by Miss Esther Bohlman.

nective tissue partitions described above into the thickness of the nerve. Inflammatory processes can extend from the eyeball toward the meningeal spaces of the brain through the spaces between the sheaths.

The optic nerve leaves the posterior pole of the eyeball in a slightly oblique direction and continues into the entrance canal of the optic nerve. Just after leaving the eye through the

THE ACCESSORY ORGANS OF THE EYE

In an early stage of embryonic development the anterior segment of the eyeball projects freely on the surface. Later a circular fold of integument encircles the cornea. From its upper and lower parts the upper and the lower lids grow toward each other over the surface of the cornea. In this way the conjunctival sac is formed

which protects and moistens the anterior free surface of the eye and especially the transparent cornea. The part lining the inner surface of the lids is the *palpebral conjunctiva*, that covering the eyeball is the *bulbar conjunctiva*. The reflection of the palpebral on the bulbar conjunctiva forms deep recesses between the lids and the eyeball, the superior and the inferior fornices. The lacrimal glands develop from invaginations of the epithelium of the conjunctiva in the region of the upper fornix.

Eyelids. Both lids have a similar structure. The outermost layer is the skin (Fig. 511). It is very thin and is provided with a few papillae and many very small hairs with sebaceous glands and small sweat glands. The derma contains a varying number of pigment cells with yellow or brown granules. The subcutaneous layer has a loose texture, is rich in fine elastic networks and in Caucasians is almost completely devoid of fat. Toward the edge of the lid the derma acquires a denser structure and has higher papillae. The horny layer of the epidermis gradually thins out.

The *eyelashes* are large hairs obliquely inserted in 3 or 4 rows along the edge of the lid (Fig. 511). With their follicles they penetrate deeply into the tissue. The shaft appears thickest in its middle and tapers down not only toward the free end, but to a certain extent also toward the root. The sebaceous glands connected with the eyelashes are very small; arrector muscles are missing. The eyelashes are replaced every one hundred to one hundred and fifty days.

Between and behind the follicles of the eyelashes, peculiar sweat glands are located, the *glands of Moll*. Unlike the ordinary sweat glands, the terminal portion here is generally straight or only slightly coiled. The excretory ducts open, as a rule, into the follicles. The epithelium of the terminal portions consists of an indistinct, outer myo-epithelial layer, adjacent to the basement membrane, and of an inner layer of pyramidal, apocrine glandular elements. The lumen is often considerably dilated and the glandular cells flattened. In the ducts the epithelium consists of two distinct cell layers. The nature of the secretion of these glands is not known.

The next layer inward consists of the thin, pale, striated fibers of the palpebral portion of the *ring muscle of the eye* (orbicular muscle). The part behind the follicles of the eyelashes or behind the ducts of the meibomian glands is the ciliary muscle of Riolan.

Behind the orbicular muscle is a layer of connective tissue, the palpebral fascia, a continuation of the tendon of the palpebral levator (or depressor) muscle. It contains the arterial arc (tarsal arc). In the upper part of the upper lid strands of smooth muscle, the *superior tarsal muscle of Müller*, are attached to the edge of the tarsus.

The *tarsus* is a plate of dense connective tissue which is the skeleton of the lid. Its oblong form corresponds to the shape of the lid. In the upper lid its breadth is about 10 mm., in the lower only 5 mm. In its substance the *glands of Meibom* are embedded (Fig. 511). They are elongated and arranged in one layer, parallel to one another and perpendicular to the length of the tarsal plate. Their openings form a single row immediately in front of the inner free edge of the lid, where the skin passes into the conjunctiva.

The meibomian glands are sebaceous, but have lobated alveolar terminal portions. Instead of being crowded at the ends of short branching ducts, they are connected by short lateral ducts with a long central excretory duct lined with stratified squamous epithelium.

The innermost layer of the lid is the *conjunctiva*. At the inner edge of the margin of the lid the epidermis continues as the narrow *admarginal zone* to the inner surface of the lid. Here the superficial cells become thicker, the number of layers decreases, mucous cells appear and the epithelium assumes a stratified columnar character which is typical for the whole conjunctiva and varies only in thickness in different places. The superficial cells have a short prismatic form and are provided with a thin cuticle. Spherical goblet cells are scattered between them.

At the upper edge of the tarsus the epithelium is sometimes reduced to two cell layers and its surface presents many irregular invaginations. Some of them are lined with mucous cells and described as glands. In the conjunctiva of the fornix the epithelium is thicker.

The lamina propria of the conjunctiva is dense connective tissue. In the region of the fornix it is loosely attached to the intra orbital fat tissue (mobile conjunctiva); this permits the free motion of the eyeball in the conjunctival sac. In its part attached to the tarsus, and also in the fornix, the lamina propria contains numerous lymphocytes and plasma cells, singly and in groups, and sometimes accumulated in small lymphoid nodules. Often lymphocytes are seen migrating into the epithelium. This infiltration is subject to great individual variations. In certain inflammatory lesions of the conjunctiva (trachoma) this infiltration may reach an enormous development and cause a thickening of the mucosa with a coarsely granular surface.

The conjunctiva of the bulb has an especially

loose lamina propria with fine elastic networks. Its deeper portion containing groups of fat cells can be looked upon as a submucous layer.

In the region of the corneal limbus the epithelium of the conjunctiva assumes a stratified squamous character and continues as such on the surface of the cornea. It may still contain a few scattered mucous cells. Its deeper cell layer in colored races contains pigment granules. The lamina propria forms distinct papillae and contains abundant elastic nets.

The rudimentary *third eyelid* or *semilunar fold* (the homologue of the nictitating membrane of the lower vertebrates) is formed by the scleral conjunctiva at the inner palpebral commissure, lateral to the lacrimal caruncle. It consists of connective tissue which contains smooth muscle fibers; it is covered with conjunctival epithelium which, on the outer surface, contains many mucous cells.

Lacrimal Gland. In connection with the conjunctival space there is a system of glands, the secretion of which moistens, lubricates, and flushes the surface of the eyeball and of the lids. Of these glands only the lacrimal gland reaches a high development. It has the size and shape of an almond and is lodged beneath the conjunctiva at the lateral upper side of the eyeball. It consists of a group of separate glandular bodies and sends out from 6 to 12 excretory ducts which open along the upper and lateral surface of the superior conjunctival fornix.

The lacrimal gland is of the tubulo-alveolar type (Fig. 545). Its terminal portions are provided with a relatively large lumen and with irregular, saccular outpocketings. The basement membrane is lined with glandular cells resembling those of the serous salivary type, as in the parotid gland. They have, however, a narrower columnar shape and contain, besides small fat droplets, large, pale secretion granules whose number changes with the volume of the cells, according to the functional conditions

Fig. 545. A small lobule of the lacrimal gland of man: *a*, Small intralobular excretory duct; *b*, terminal portions, *c*, intralobular interstitial connective tissue with blood vessels; *f*, fat cells; *A*, cross section of a larger interlobular excretory duct with pseudostratified epithelium. 112 ×. After Schaffer.

These cells are provided with secretory capillaries; between their bases and the basement membrane well developed basket (myo epithelial) cells are present. The smallest intralobular excretory ducts are lined with a layer of low columnar or cuboidal cells and have a few myoepithelial cells. The larger intralobular ducts have a two-layered epithelium.

On the inner surface of the lids, especially the upper one, near the upper edge of the tarsus, a varying number of small accessory lacrimal glands—the *tarsal lacrimal glands*—are scattered (Fig. 544, *k*).

After having washed the conjunctival cavity, the secretion of the lacrimal gland (the tears,

a sterile liquid) reaches the region of the inner palpebral commissure (internal canthus). Here the two eyelids are separated by a triangular space, *the lacrimal lake*, in which the secretion accumulates temporarily. From here it passes through two tiny orifices called *lacrimal points*, one on the margin of each eyelid, into the *lacrimal ducts*. The latter converge medially into the *lacrimal sac* whence the *nasolacrimal* duct leads into the inferior meatus of the nose.

The wall of the excretory lacrimal passages is formed by connective tissue lined with epithelium The first is a continuation of the lamina propria of the conjunctiva and of the nasal mucosa. In the lacrimal ducts and sac it contains abundant elastic nets and many lymphoid elements. In the lower end of the nasolacrimal duct it is surrounded by a cavernous venous plexus. The epithelium of the lacrimal ducts is stratified squamous. The lacrimal sac and the nasolacrimal duct are lined with a pseudostratified, tall columnar epithelium.

From the bottom of the lacrimal lake, between the two lacrimal ducts, there bulges a small, elongated, soft mass of tissue, the *lacrimal caruncle*. Its structure presents a peculiar mixture of features typical for the skin and for the conjunctiva. The top is covered with a thick, squamous epithelium in which only the uppermost layers are flattened, although not cornified. It contains mucous cells. On passing down the slopes this epithelium gradually assumes the character of common conjunctival epithelium. The lamina propria is covered with papillae and contains lymphocytes, bundles of striated muscles, sweat and abortive lacrimal glands; besides, there are tiny hairs with sebaceous glands. These are the source of the whitish secretion which often collects in the region of the inner palpebral commissure.

Blood and Lymph Vessels of the Eyelids. The arteries in each lid form two archlike anastomoses, which run in front of the tarsus, one near the free margin of the lid, the other near the upper (or lower) margin of the tarsus. The palpebral conjunctiva is provided with dense, subepithelial capillary networks which can be easily studied in living condition with the aid of the slit-lamp microscope. Branches of the blood vessels in the scleral conjunctiva anastomose with the marginal blood vessels of the cornea and with the branches of the anterior ciliary arteries.

The lymphatics form dense nets in the conjunctiva behind the tarsus. In front of the latter there is another, thinner, pretarsal net. A third net can be distinguished in the skin and the subcutis. All these nets communicate with one another. The lymphatic capillaries of the scleral conjunctiva end blindly near the corneal margin.

The abundant supply of the conjunctiva with blood and lymph capillaries explains the rapid absorption of solutions introduced into the conjunctival sac.

The Histogenesis of the Eye. The first stages of the development of the primary and the secondary optic vesicles from the brain have already been mentioned (pp. 600, 613). It has also been explained that, while the stalk of the vesicle is transformed into the optic nerve, the double-walled vesicle gives rise to the retina with all its parts.

Where the lateral wall of the optic vesicle touches the ectoderm, the epithelium of the latter forms an invagination with a greatly thickened bottom, the *primordium of the lens* (Fig. 516, *LE, LF*). It apparently develops as the result of a peculiar stimulation exerted by the optic vesicle upon the ectoderm. In amphibian larvae, after excision of the optic vesicle, the lens is not formed.

The lens primordium comes to be in the invagination of the optic vesicle. Simultaneously, mesenchyme and blood vessels grow into the chorioidal fissure which splits the lower periphery of the vesicle and continues upon the optic stalk. These vessels give rise to the hyaloid and retinal vascular systems. The opposite margins of the fissure, which received the vessels, soon grow together and the secondary optic vesicle assumes the form of a double-walled cup, while the stalk is transformed into a solid strand, the optic nerve.

The lens primordium soon becomes detached from the ectoderm and the space between the two is filled by the layer of mesenchyme: *the primordium of the substantia propria of the cornea and of the connective tissue of the iris* (Fig. 516, *M*). The lens, surrounded by vascular mesenchyme, acquires a solid, spherical form, while the original cavity disappears.

The inner, thicker sheet of the double wall of the optic cup differentiates into the *retina proper* (Fig. 546, *IB*, layers 2 to *10* in Fig. 531); it remains permanently in direct continuation with the optic nerve. The outer, thinner sheet of the cup is transformed into the pigment epithelium (layer *1* in Fig. 531).

The surrounding mesenchyme comes into close relation with the optic cup and gives rise to the two outer tunics of the eyeball, the *uveal* and *fibrous tunics*.

The structural differentiation of the *retina* proceeds in a way similar to that of the wall of

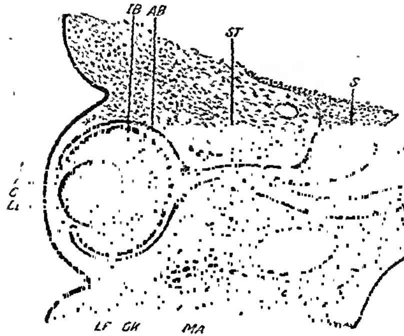

Fig. 546. Primordium of eye of a mouse embryo of 8 mm. The cavity of the primary optic vesicle is reduced to a thin cleft; *AB*, outer layer of secondary optic vesicle; *B*, bottom of anterior brain vesicle; *CE*, epithelium of cornea; *GK*, vitreous body; *IB*, inner layer of secondary optic vesicle; *LE*, epithelium of the lens; *LF*, lens fibers with nuclear zone; *M*, mesenchyme; *MA*, primordium of muscle; *S*, side of anterior brain vesicle; *ST*, stalk of optic vesicle; *U*, border of the optic cup; *V*, ventricle of brain. 70 ×. After Schaffer.

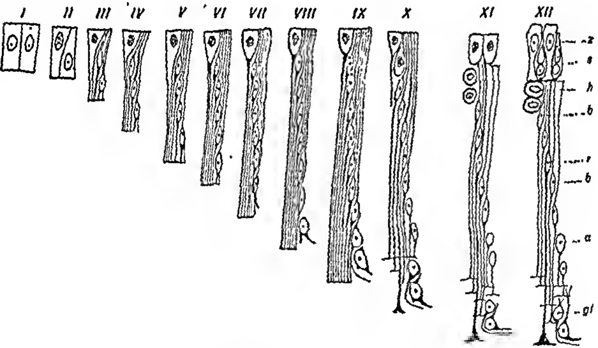

Fig. 547. Diagram of the histogenesis of the retina. *I*, Simple regular columnar epithelium; *II*, pseudostratified epithelium with two and, *III*, with three rows of nuclei; the number of nuclear rows increases in *IV–VII*; *VII*, the lowermost cell has developed an axon; *VIII*, the first (lower) ganglion cell has separated from the other elements; *IX*, the second ganglionic cell becomes separated; *X*, all cells except the radial fibers are separated from the inner surface; the ganglion cells and the amacrines have also severed their connections with the outer surface; *XI*, all cells except the indifferent stem cells, the visual cells, and the radial fibers are separated from the outer surface; the horizontal cells also are free; *XII*, the rod cells (*s*) form a double layer between the cone cells; the cones possess protoplasmic outgrowths; *z*, cone cells; *h*, horizontal cells; *r*, radial fibers; *b*, bipolars; *a*, amacrines; *gl*, ganglion cells. After Furst from Franz.

the neural tube (p. 215). It is characterized by proliferation, by shifting of the cells and by the establishment of complex synaptic relationships (Fig. 517).

The eyeball attains full size toward the end of the first decade, whereas the structure of the retina, including the central fovea, matures toward the end of the first year.

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Chapter XXVIII

THE EAR

THE organ of hearing consists of three parts. The first part, the *external ear*, receives the sound waves; the second, the *middle ear*, transmits the vibrations to the third part, the *internal ear* or the labyrinth, where the sound waves elicit specific nervous impulses. These are conveyed by the acoustic nerve to the central nervous system. The internal ear also contains the vestibular organs which are highly specialized end organs of the proprioceptive sense and are concerned chiefly with the function of equilibration.

THE EXTERNAL EAR

The external ear includes the auricle (concha or pinna), the external acoustic meatus, and the tympanic membrane.

The Auricle. The complicated form of the auricle is caused by its irregular plate of elastic cartilage, 0.5 to 1 mm. thick. It contains very numerous cells and but scanty intercellular substance; it is surrounded by a firm and flexible perichondrium with abundant elastic networks The skin covering the auricle is provided with a distinct subcutaneous layer only on the posterior, convex surface. It carries a few small hairs with sebaceous glands, sometimes of considerable size; in old age, especially in men, large stiff hairs develop at the dorsal edge and at the ear lobe. The sweat glands are scarce and small.

The External Auditory Meatus. The external auditory meatus has an oval cross section and extends from the bottom of the auricle to the tympanic membrane which closes its inner end and separates it from the tympanic cavity. Its walls are formed by the continuation of the cartilage of the auricle in its outer part and by the temporal bone in its inner part. The skin lining its wall is thin, devoid of papillae, firmly attached to the perichondrium and periosteum and has no subcutaneous layer. In the outer, cartilaginous portion numerous hairs are present which protect the meatus against the entrance of foreign bodies. In old age they enlarge considerably in the same way as the hairs of the auricle. The sebaceous glands connected with the hair follicles are exceptionally large. In the inner, osseous portion small hairs and sebaceous glands are found only along the upper wall.

A typical feature of the external meatus is a peculiar secretion— the *cerumen*— a brown, waxlike mass with a bitter taste, which protects the skin from desiccation and from invasion by insects. It is a mixture of the secretion of the sebaceous glands just mentioned and of peculiar, large, ceruminous glands which are also found in the skin of the meatus. They are of the tubular, coiled type and are a variety of the apocrine sweat glands. The ducts of the ceruminous glands open either directly on the free surface of the skin of the meatus or, together with the sebaceous glands, into the necks of the hair follicles.

The Tympanic Membrane. The oval tympanic membrane is thin and semitransparent. One of the auditory ossicles,

the malleus (see p. 638), is attached by its manubrium (the handle), to the inner surface of the membrane and reaches its center.

The main mass of substantia propria of the tympanic membrane is formed by *two* layers of collagenous bundles similar to those of a tendon. The fibers (Fig. 518, *f*) in the outer layer have a radial arrangement. The inner layer consists of circular collagenous fibers. Fibroblasts are scattered between the fibers of both layers; *there are also thin networks of elastic* fibers which are more conspicuous in the central part of the membrane and at its periphery.

On its outer surface the substantia propria is lined by a very thin (50–60 μ) layer of skin, on its inner surface by the mucous membrane of the tympanic cavity which in this region is reduced to only 20 to 40 μ in thickness. The derma of the skin blends with the radial fibers and has no papillae. The epidermis has a thin, loose, horny layer (Fig 518, *e'*). Along the handle of the malleus the skin is slightly thickened and provided with a layer of subcutaneous tissue, through which the vessels and nerves reach the center of the membrane. The lamina propria of the mucosa is represented only by a few thin collagenous fibers and blood capillaries and is covered by a layer of *simple squamous epithelium.*

THE MIDDLE EAR

The middle ear comprises the tympanic cavity and the auditory or *eustachian tube.*

The Tympanic Cavity. The tympanic cavity is an irregular, air-containing space within the substance of the temporal bone. Its lateral wall is largely formed by the tympanic *membrane*; the *medial wall* by the lateral side of the osseous labyrinth (see following pages). It contains the ear bones or auditory *ossicles*, with their articulations and ligaments; two small muscles, tensor tympani and stapedius, partly enclosed in bony tubes and connected with the ossicles; the chorda tympani nerve; and connective tissue trabeculae. The tympanic cavity continues into the auditory tube, which opens into the *nasal part* of the pharynx. It is derived from the first branchial pouch of the embryo.

Fig. 518. Cross section of the edge of the tympanic membrane of a child: *a*, Fibrocartilaginous ring; *b*, bone, *c*, derma with papillae; *d*, mucous membrane of tympanic cavity, *e*, epidermis of the external meatus; *e'*, epidermis of the tympanic membrane; *f*, radial fibers; *f'*, circular fibers of the tympanic membrane; *g*, *mucosa* of the tympanic membrane; *h'*, its squamous epithelium, *h*, ciliated columnar epithelium of the tympanic cavity. *i*, vessels. Redrawn from v. Ebner.

In the adult, the posterior part of the tympanic cavity is connected, through the large tympanic antrum, with the air-filled cavities, or "cells," in the mastoid process of the temporal bone.

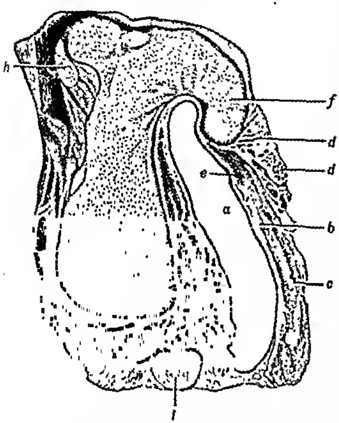

Fig. 519. Transection of the cartilaginous portion of the auditory tube near its opening into the pharynx. *a*, Lumen of the tube in dilated condition; *b*, ciliated epithelium; *c*, membranous lateral wall with fat tissue; *d*, muscle bundles; *e*, mixed glands; *f*, lateral cartilaginous plate forming hook; *g*, medial cartilaginous plate with darker spots caused by patches of elastic cartilage. *h* and *i*, accessory cartilages. 11 ×. Redrawn from v. Ebner

The epithelium of the tympanic cavity is generally of the simple squamous type; in several places, especially near the opening of the auditory tube, and near the edge of the tympanic membrane, it is cuboidal or columnar and provided with cilia. The existence of glands is generally denied.

On the medial wall of the tympanic cavity, formed by the osseous labyrinth, are two "windows," the fenestrae. One of these, the *vestibular fenestra*, is an oval opening which is closed by the base of the stapes; this is attached to the cartilaginous edges of the opening by means of a circular fibroblastic ligament. This fenestra ovalis separates the tympanic cavity from the scala vestibuli of the cochlea. The other opening is round—the *fenestra tympanica* or *rotunda*. It is situated below and behind the oval fenestra and is closed by a slightly concave, fibrous membrane (secondary tympanic membrane). It separates the tympanic cavity from the scala tympani of the cochlea (see below).

Auditory Ossicles. There are three auditory ossicles—the malleus (hammer), the incus (anvil), and the stapes (stirrup). They form a chain extending from the tympanic membrane, to which the malleus is attached, to the oval fenestra, which is closed by the base of the stapes; they are connected with each other by tiny articulations. Their substance is bone, which contains small cavities with blood vessels and connective tissue. On the handle of the malleus and the base of the stapes are small patches of hyaline cartilage. The periosteum covering the ossicles fuses with the lamina propria of the mucous membrane into a very thin layer of connective tissue which is covered by simple squamous epithelium.

The Auditory or Eustachian Tube. The auditory tube has a small flattened lumen (1 to 2 mm.) which in the section nearest to the tympanic cavity is surrounded by bone. In the following part the wall is supported by a groovelike plate of hyaline cartilage. The mucous membrane which lines the lumen in the bony portion has a low columnar ciliated epithelium; in the portion nearer the pharynx a taller, *pseudostratified ciliated epithelium* is found; at the pharyngeal opening numerous goblet cells appear. A function of the tympanic cavity is to regulate the air pressure on the inner side of the tympanic membrane. By the act of swallowing, which is periodically repeated, the lumen of the tube is opened for short intervals

and the air pressure in the middle ear is equalized with the outside pressure.

THE INNER EAR OR LABYRINTH

The inner ear is called the labyrinth because of its complex structure. It is enclosed in the petrous part of the temporal bone. It comprises a series of canals and cavities which are hollowed out of the bone and are known as the osseous labyrinth.

Osseous Labyrinth. As the layer of bone immediately surrounding the cavities is harder than the rest of the petrous portion, especially in the infant, it is possible, by careful dissection, to isolate the osseous labyrinth from the mass of the bone. It must be kept in mind, however, that this aspect of the free osseous labyrinth is entirely artificial.

In the osseous labyrinth, artificially isolated from the temporal bone, a central part of irregular, oval shape can be distinguished—the *vestibule.* It is situated medial to the tympanic cavity. Its lateral wall, which faces the tympanic cavity, has the two fenestrae mentioned above.

The three *semicircular canals* are loop-shaped, long tubes which describe the greater part of a circle; they arise from above and behind the vestibule and return to it. According to their position in space relative to the skull they are distinguished as the superior or frontal, the posterior or sagittal, and the lateral or horizontal canal. The lateral canal is the shortest of the three (12–15 mm.); the posterior is the longest (18–22 mm.). The lateral canals of both ears are situated very nearly in the same plane. The superior canal of one side is approximately parallel to the posterior canal of the other.

The lateral end of the superior canal and the anterior end of the lateral canal present dilatations, called the *ampullae,* which open near each other into the upper part of the vestibule above the oval fenestra. The ampullated lower end of the posterior canal opens into the lower posterior part of the vestibule. The opposite (upper) end of this canal fuses with the medial end of the superior canal to form a common stem, the crus commune, which opens into the upper medial part of the vestibule. The posterior end of the lateral canal opens independently into the upper, posterior part of the vestibule. From the medial wall of the vestibule a thin osseous canal extends to the posterior surface of the pars petrosa of the temporal bone—the *vestibular aqueduct.*

The anterior periphery of the vestibule continues into the *bony cochlea*—a spirally coiled tube, which forms a conical body resembling a snail shell. Its apex is directed forward, laterally and slightly downward. It measures about 5 mm. from base to apex with a diameter of about 9 mm. at the base.

Membranous Labyrinth. The interior of the osseous cavities is lined with a layer of periosteum and encloses a system of vesicles and canals with a fibrous wall—the membranous labyrinth. All parts of the latter are continuous and communicate with one another; they are filled with a clear fluid, the *endolymph.* The inner surface of their fibrous walls is lined with an epithelium of ectodermal origin. The outer surface of the fibrous walls of the membranous labyrinth in some places adheres to the periosteum of the osseous labyrinth. In general, however, it is separated from this periosteum by large or small, irregular cavities filled with a clear liquid, the *perilymph.* Thin, irregular strands or membranes of connective tissue (trabeculae) arise from the periosteum, penetrate the perilymphatic spaces and reach the wall of the membranous labyrinth, together with the blood vessels. Thus, the membranous labyrinth is suspended within the osseous labyrinth by these trabeculae. The perilymphatic spaces are homologous to the subarachnoid spaces of the meninges; the perilymph corresponds with the cerebrospinal fluid.

The form and arrangement of the various parts of the membranous labyrinth generally correspond with those of the osseous labyrinth in which they are enclosed. However, the membranous part contained in the osseous vestibule consists not of one, but of two sharply separated sacs—the utricle and the saccule.

It is relatively easy to isolate the membranous labyrinth from within its osseous container. In such a free condition it presents the following parts:

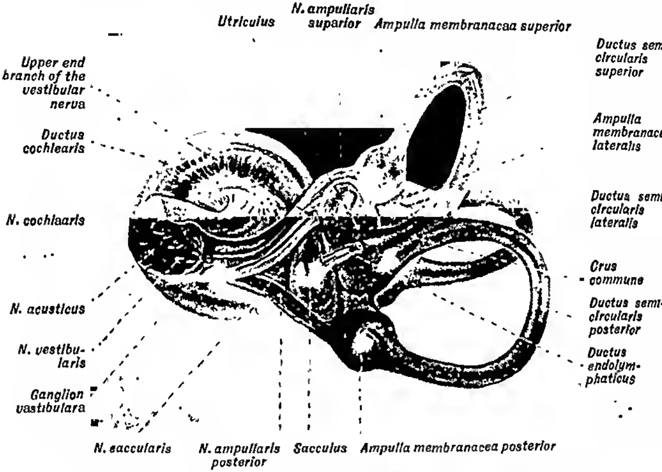

Fig. 550. Right membranous labyrinth of an adult; medial and posterior aspects. 5 ×. After Spalteholz.

The central position is occupied by the utricle and the saccule (Fig. 550). The *utricle,* or elliptical sac, has an oblong, transversely compressed form and is the larger of the two. It occupies the upper, posterior part of the osseous vestibule and communicates with the three membranous semicircular canals by five orifices. These canals lie in the three dimensions of space and correspond exactly in form and position with the osseous canals in which they are contained. The three ampullar enlargements are much more pronounced in the membranous canals. Each ampulla has a flattened floor which forms part of the convex surface of the respective canal and a hemispherical roof bulging on the concave side. The superior and lateral ampullae open, very close to each other, into the upper end of the utricle; the posterior ampulla into its lower end. The *crus commune* formed by the superior and inferior canals joins the middle part of the utricle in the vicinity of the second orifice of the lateral canal.

The approximately *spherical saccule* or round sac lies in front of and is connected with the utricle by the utriculosaccular duct; this consists of two convergent parts arising from both sacs. Their junction continues as a slender canal, the *endolymphatic duct,* which runs through the vestibular aqueduct to the posterior surface of the petrous part of the temporal bone

where it ends with a bulblike enlargement, the *endolymphatic sac*.

From the lower part of the saccule a short, narrow duct, the *ductus reuniens* (Fig. 551, *g*) leads to the membranous cochlea or the cochlear duct.

The wall of the membranous labyrinth undergoes important modifications and acquires an extremely complex structure in certain well outlined sensory areas which are formed by thickened epithelium and contain the endings of the acoustic nerve. There are six such neuro-epithelial areas in each labyrinth (Fig. 551). Two of these areas, the maculae, lie in the utricle and the saccule—the *macula utriculi* and *macula sacculi*. Other neuro-epithelial areas are the three *cristae ampullares*, one in the ampulla of each semicircular canal. The sixth neuro-epithelial area, the largest and most complex of all, the *organ of Corti*, is a thick ridge which runs along the cochlear canal.

The Utricle and Saccule. The connective tissue layer of the wall of the utricle and saccule, as well as of all the other parts of the membranous labyrinth, consists of a finely fibrillated, intercellular substance and of spindle-shaped or stellate fibroblastic cells. Branched melanophores are often encountered in this tissue. Its outer surface, the trabeculae which run through the perilymphatic spaces, and the inner surface of the periosteum, are everywhere lined with flattened connective tissue cells forming a mesenchymal epithelium.

The connective tissue is separated from the epithelium by a basement membrane. Outside the neuro-epithelial areas the epithelium consists of one layer of polygonal squamous cells. They are 3 to 4 μ thick and are usually provided with a diplosome and a flagellum.

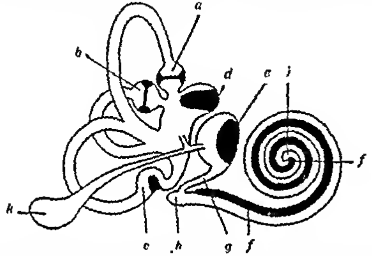

Fig. 551. Diagram of the left membranous labyrinth as seen from within. Neuro-epithelial areas black. *a*, Superior; *b*, lateral; *c*, posterior ampullae of the respective semicircular canals; *d*, macula utriculi; *e*, macula sacculi; *ff*, organ of Corti in the cochlear duct; *g*, ductus reuniens; *h*, cecum vestibulare; *i*, cecum cupulare; *k*, saccus endolymphaticus, continuing into the ductus endolymphaticus; the latter opens into the utriculosaccular duct. Redrawn after v. Ebner from Schaffer, slightly modified.

The *macula utriculi* occupies the lateral wall of the utricle, has an oval, spoon-shaped form and measures 2 x 3 mm. The macula sacculi occupies the medial wall of the saccule. It is of similar size and is heart shaped. The surface of the utricular macula lies approximately in the plane of the base of the skull (and of the lateral or horizontal semicircular canal). The surface of the saccular macula follows, at least in part, the sagittal plane. Thus, the surfaces of both maculae are perpendicular to each other.

In both maculae the epithelium has the

The form and arrangement of the various parts of the membranous labyrinth generally correspond with those of the osseous labyrinth in which they are enclosed. However, the membranous part contained in the osseous vestibule consists not of one, but of two sharply separated sacs—the utricle and the saccule.

It is relatively easy to isolate the membranous labyrinth from within its osseous container. In such a free condition it presents the following parts:

The central position is occupied by the utricle and the saccule (Fig. 550). The *utricle*, or elliptical sac, has an oblong, transversely compressed form and is the larger of the two. It occupies the upper, posterior part of the osseous vestibule and communicates with the three membranous semicircular canals by five orifices. These canals lie in the three dimensions of space and correspond exactly in form and position with the osseous canals in which they are contained. The three ampullar enlargements are much more pronounced in the membranous canals. Each ampulla has a flattened floor which forms part of the convex surface of the respective canal and a hemispherical roof bulging on the concave side. The superior and lateral ampullae open, very close to each other, into the upper end of the utricle; the posterior ampulla into its lower end. The *crus commune* formed by the superior and inferior canals joins the middle part of the utricle in the vicinity of the second orifice of the lateral canal.

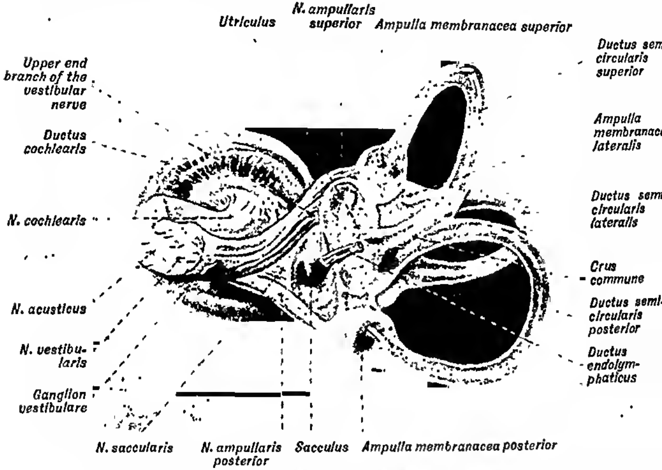

Fig. 550. Right membranous labyrinth of an adult; medial and posterior aspects. 5 ×. After Spalteholz.

The approximately *spherical saccule* or round sac lies in front of and is connected with the utricle by the utriculosaccular duct; this consists of two convergent parts arising from both sacs. Their junction continues as a slender canal, the *endolymphatic duct,* which runs through the vestibular aqueduct to the posterior surface of the petrous part of the temporal bone

the *crista* presents itself in cross section. as a high, rounded prominence occupying about one third of the lumen. In a cross section through the ampulla the crista is cut longitudinally and is seen to be highest in its middle part and to slope down toward the side walls of the ampulla. The ends of the crista, as seen from the surface, after the roof of the ampulla is cut open, are rounded off and edged by a crescentic area, the *planum semilunatum.* The latter is covered by a columnar epithelium which contains inclusions and has, perhaps, a glandular function.

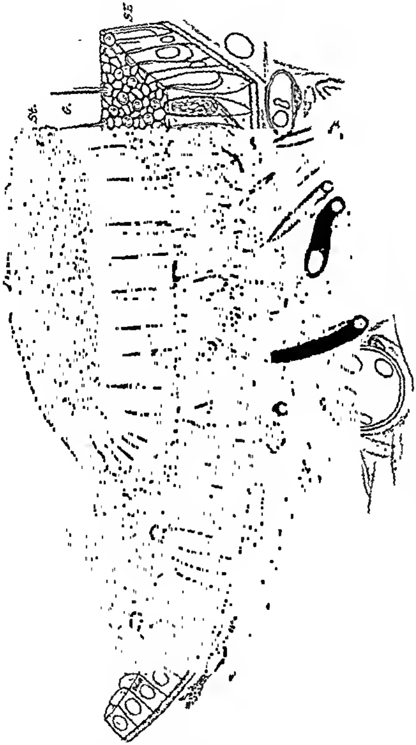

Fig. 552. Plastic diagram of the border region of a macula: *UE*, Transitional epithelium; *St*, otoconia; *G*, gelatinous layer; *SE*, sustentacular elements. After Kolmer.

Profound changes are made in the crista during fixation and the details of the living structure of the human ear are unknown. In fishes, as described by Bowen, the living neuro-epithelium

same structure. It is 30 to 35 μ thick and consists of two kinds of elements—the *supporting* or *sustentacular cells* and the *hair cells*. The first are slender columnar structures with a protoplasm containing a bundle of rigid tonofibrils and a round nucleus at the lower end. The free surfaces are provided with cuticular plates and are connected with one another by a system of terminal bars. Under each cuticular plate lies a diplosome with a minute protruding flagellum and, farther downward, a Golgi net and sometimes granular or fatty inclusions.

The *hair cells* are very difficult to fix. They are lodged between the supporting cells, but occupy only the upper half of the epithelial layer and do not reach the basement membrane. They have the form of short flasks with a round bottom which contains the nucleus and are covered on their free surface with a round, cuticular plate. The latter is connected with the cuticles of the supporting cells. From the center of the cuticle rises a tuft of long (20 to 25 μ), very thin, nonmotile cilia, which are kept together by means of a cement substance and thus form a long, tapering, stiff brush. The diplosome is also present under the cuticle; it sends out a flagellum which adheres to the surface of the tuft of cilia and is believed to beat during life. Above the nucleus a Golgi net and mitochondria have been described. The intercellular spaces between the supporting cells and the hair cells are filled with a peculiar semifluid substance.

The surface of the maculae is covered by the *otolithic membrane*—a thick (22 μ) layer of a gelatinous substance into which the hair tufts penetrate. Each tuft is surrounded by a narrow tubular space filled with endolymph. Between these spaces the gelatinous substance is connected with the terminal bars of the epithelium by means of thin partitions. The upper layer of the jelly, beyond the ends of the hairs, contains a multitude of minute (3×5 μ) crystalline bodies, the *otoconia* or *otoliths* (Fig. 552, *St*). They have the form of a prism ending in pyramids and are a mixture of calcium carbonate (aragonite) and a protein. After the calcium is dissolved by acids, their outlines remain visible. Owing to the reflection of light by the otoconia, the maculae on macroscopic examination in fresh condition present an opaque, white aspect.

At the edge of the macula the row of hair cells is abruptly discontinued, while the supporting cells gradually pass into the simple squamous epithelium of the rest of the wall.

The connective tissue of the wall is thickened in the area of the macula and is firmly attached to the endosteum. Here the intercellular substance has an especially firm, cartilaginous consistency, and the basement membrane is very distinct. Most of the myelinated nerve fibers which supply the macula lose their myelin sheaths in the immediate neighborhood of the basement membrane. The naked axis cylinders, among which thick and thin fibers can be distinguished, pierce the membrane and branch in the intercellular spaces between the epithelial cells. The terminal arborizations of the thick fibers form basket-like nests, closely surrounding the surface of the hair cells and almost reaching the free epithelial surface. The thin fibers end with free branches between the supporting cells.

The Semicircular Canals. The membranous semicircular canals have a slightly oval cross section. They occupy an eccentric position in the osseous canals. Their convex surface is closely adjacent to the periosteum while their concave surface is surrounded by a large perilymphatic space (Fig. 553, *pr*) with numerous trabeculae (Fig. 553, *b*). The wall of the membranous canals has the same structure as the wall of the utricle and saccule.

In a longitudinal section of the ampulla

arrangement of the parts of the cochlea in space. Its conical body is supposed to stand with its axis upright, its base below and its apex or cupula above. The radial direction from the axis toward the surface of the cone is called outward, the radial direction toward the axis inward. The direction parallel to the course of the canal is termed spiral. A plane parallel to the axis but not passing through it may be designated as tangential. The most convenient sections for the histologic study of the cochlea are radial ones which pass through the plane of the axis and perpendicularly to the cochlear canal.

The *axis of the cochlea*, as seen in a radial section, *is represented by a broad* and short, conical pillar of spongy bone, the modiolus. Its base forms the bottom of the internal acoustic meatus. Blood vessels surrounded by abundant connective tissue and bundles of the cochlear division of the acoustic nerve penetrate through numerous openings into the bony substance of the modiolus. The nerve fibers run upward and successively turn outward to

Fig. 554 Plastic diagram of one half of an ampullar crista as seen in a longitudinal section of a semicircular canal, passing across the crista: *SH.*, Hair tufts; *C.*, gelatinous mass of the cupula; *UE*, transitional epithelium; *Pl.sem.*, planum semilunatum After Kolmer

of the crista is clothed with flexible sensory hairs of uniform length (50 μ in the catfish) which show spontaneous movements. When reagents are applied to the living crista under the microscope the hairs are seen to shorten slowly and the space in the lumen which they formerly occupied is filled with a coagulum which is ultimately condensed and shrunken to the form of the cupula as described below. The cupula of fixed preparations is therefore an artefact.

In well fixed preparations the neuro-epithelium of the human crista has much in common with that of the maculae, with similar supporting elements and hair cells. The remaining basal portions of the hairs may be 30 μ in length. Near the surface of each hair cell is a diplosome from which a flagellum arises and the latter is continuous with what appears to be a tuft of cilia matted together. The crista is covered by a cap or bell-like gelatinous mass, the cupula. Between the surface of the epithelium and the cupula there is a narrow space filled with endolymph. The hairs, before penetrating into the cupula, have to pass through this space. Thus, the cupula seems to be supported by the hairs and to rest upon them. On its way through the gelatinous mass of the cupula, each of the hairs, as in the maculae, is surrounded by a narrow canal filled with endolymph. In cross sections of the crista the cupula usually presents a regular tangential striation. The relations of the nerve fibers to the hair cells are essentially the same as in the maculae.

Fig. 553. Cross section of the lateral semicircular canal of an adult man: *b*, Connective tissue trabeculae in the perilymphatic space; *e*, epithelium; *en*, endolymphatic space; *g*, blood vessel; *k*, bone of the bony labyrinth; *ks*, bone trabeculae of the spongiosa; *l*, ligamentum canaliculi; *m*, membrana propria of the membranous semicircular canal; *n*, bone marrow; *p*, periosteum; *pr*, perilymphatic space. 46 ×. After v. Ebner, from Schaffer.

The Cochlea. The canal of the cochlea takes two and a half spiral turns around the axis of this structure. For the sake of a convenient description it is necessary to define certain terms regarding the general

ous and the whole of the membranous spiral lamina; the upper wall is formed by the vestibular membrane, and the outer wall by the wall of the osseous cochlear canal. In the lowest coil the vestibular membrane forms with the spiral lamina an angle of about 45 degrees. In the upper coils the angle becomes smaller, its apex is rounded off and the height of the cochlear duct decreases.

The scala vestibuli extends into and through the perilymphatic space of the vestibule and thus reaches the inner surface of the fenestra ovalis. At the apex of the cochlea the two scalae communicate with each other through a minute opening—the *helicotrema.*

The lower, vestibular end of the cochlear duct is a small, blind outpocketing and is called the *cecum vestibulare;* it is separated from the fenestra ovalis by the enlarged perilymphatic space. Into it opens the above-mentioned canalis reuniens, which connects it with the saccule; in adults it is almost obliterated. The upper end of the cochlear duct ends blindly with the cecum cupulare or lagena, close to the helicotrema.

The functions of the structures in the cochlea are so imperfectly known that no consistent and generally accepted theory has been formulated.

The Scalae. The structure of the wall of the two scalae corresponds to what was described for the perilymphatic spaces in the other parts of the labyrinth. The bone is lined by a thin periosteal (or endosteal) layer of connective tissue covered with mesenchymal epithelium.

The Osseous Spiral Lamina and the Basilar Membrane. The spiral ganglion extends along the line of attachment of the osseous spiral lamina to the modiolus. It is lodged in an irregular cavity of the bone, the *spiral canal of the modiolus.* From the ganglion, along its whole length, bundles of nerves arise and run in the radial, outward direction, through radial canals in the osseous spiral lamina, toward the organ of Corti.

In the inner corner of the cochlear duct the periosteum of the upper surface of the spiral lamina forms a ridgelike prominence bulging into the duct—the *limbus spiralis.* In the inward direction it gradually slopes down. Its higher outer edge falls away abruptly and forms a sharp crest which overhangs a groove open to the periphery—the *internal spiral sulcus* (Fig. 556). In a radial section this concavity has the shape of the letter C. The two margins of the sulcus are called the *vestibular* and the *tympanic lip* or labium.

The connective tissue of the limbus has a peculiar, firm, almost homogeneous, intercellular substance and contains, especially in its deeper layers, stellate connective tissue cells. On the surface the connective tissue presents deep radial furrows with small, ridgelike prominences, between them. When seen from the upper surface, the latter protrude over the edge of the vestibular lip in the form of teethlike processes—the *auditory teeth of Huschke.*

The periosteum on the lower surface of the spiral osseous lamina continues outward beyond the tympanic lip of the limbus. Its thickness at this place shows great individual variations. The tympanic lip, in the major part of the cochlea, also consists of connective tissue which is the continuation of the tissue of the limbus and has the same structure. It contains the radial bundles of myelinated fibers which come from the spiral ganglion through the osseous lamina. In the lower coil of the cochlea the osseous lamina extends outward as far as the tympanic lip.

A little beyond the vestibular lip, the nerve bundles leave the connective tissue (or, in the lower coil, the osseous lamina) and enter the epithelium of the organ of Corti. In doing so, they emerge from the connective tissue (or from the bone) through a series of small radial slits, the foramina nervosa. When seen from the

reach the spiral ganglion which extends along the inner wall of the cochlear canal.

The lumen of the canal of the osseous cochlea (about 3 mm. in diameter) is divided along its whole course (about 35 mm. in man) into an upper and a lower section by a spiral partition, the *spiral lamina*, which gradually becomes broader toward the upper part of the cochlea. The inner zone of the partition consists of bone and is called the *osseous spiral lamina* (Fig. 555, *Lo*); it projects from the modiolus (in macerated specimens) in the form of a shelf and ends at the apex of the cochlea in a hooklike projection, the *hamulus*. The outer zone is fibrous and is called the *membranous spiral lamina* or the *basilar membrane* (Fig. 555, *Mb*). The relative width of both zones, *i. e.*, the extent of the ossification of the spiral partition, varies according to the level of the cochlea; it is greatest in the lowest coil.

At the line of attachment of the basilar membrane to the outer wall of the cochlea the periosteum is greatly thickened and forms, in a radial section, a triangular mass of connective tissue, the *spiral ligament* (Fig. 555, *Ls*).

From the upper surface of the inner zone of the spiral lamina the thin vestibular membrane (*membrane of Reissner*) (Fig. 555, *Mv*) extends obliquely to the outer wall of the canal of the osseous cochlea and forms a more or less acute angle with the spiral lamina. Thus, the cross section of the osseous cochlear canal will show three cavities: the upper cavity or *scala vestibuli* is surrounded by the osseous spiral lamina, the vestibular membrane and the upper periphery of the osseous wall. The lower cavity or *scala tympani* is bounded by both the osseous and the membranous spiral laminae, and by the lower periphery of the osseous wall. Both scalae are perilymphatic spaces. Between them is the *ductus cochlearis* (*scala media or membranous cochlear canal*); it is triangular in transection, with the acute angle directed inward. Its lower wall is formed by a part of the osse-

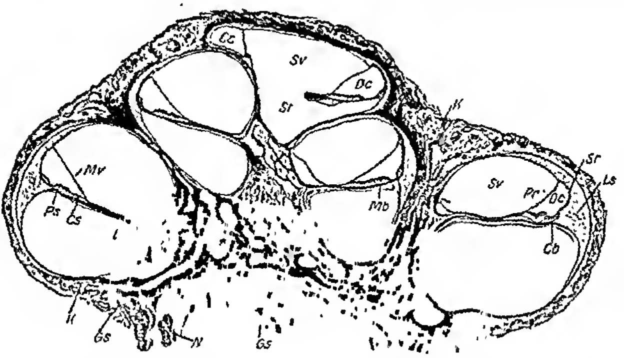

Fig. 555. Axial section of the cochlea of a man: *Cb*, Crista basilaris; *Cc*, cecum cupulare; *Cs*, crista spiralis; *Dc*, ductus cochlearis; *Gs*, ganglion spirale; *K*, bony wall of the cochlea; *Lo*, lamina spiralis ossea; *Ls*, ligamentum spirale; *Mb*, membrana basilaris; *Mv*, membrana vestibularis; *N*, cochlear nerve; *Pr*, prominentia spiralis; *Ps*, organ of Corti; *Sr*, stria vascularis; *St*, scala tympani; *Sv*, scala vestibuli. 16 ×. After Schaffer.

this lip and the crest of the spiral ligament. The basilar membrane can be subdivided into an inner zone (zona arcuata) extending from the foramina nervosa to the base of the external pillars, and an outer zone (zona pectinata) between the external pillars and the crest of the spiral ligament.

The middle layer in both zones is formed by peculiar fibers, the *auditory strings* or *basilar fibers*. In the zona arcuata they are thin and arranged in the fashion of a net; in the zona pectinata they are thicker (1 to 2 μ), straight and smooth and do not branch.

In fresh condition they are soft and flexible and can be easily isolated. Acetic acid dissolves them; after fixation they become hard and brittle. They are birefringent, but differ from collagenous as well as from elastic fibers. They are embedded in a small amount of a homogeneous ground substance. On reaching the spiral ligament some of the strings run upward under the epithelium. Others penetrate fanlike into the tissue of the ligament.

The length of the strings increases considerably from the base of the cochlea to its apex and the range of this variation differs in individuals. In the beginning of the first coil they have a length of 64 to 128 μ; at the end of the membrane, at the hamulus, they measure 352 to 480 μ. The total number of the strings in the basilar membrane of the human cochlea is estimated at 24,000.

On the upper surface of the middle layer with its auditory strings there is a thin, homogeneous, upper or *vestibular covering layer* with a few radially arranged connective tissue nuclei. It can be isolated in material fixed with osmic acid. The lower surface is lined with the *tympanic covering layer*. It is delicate connective tissue which in some places may present thickenings bulging into the scala tympani and which consists of a very loose intercellular substance and of cells stretched in the spiral direction. This layer contains blood capillaries in its inner zone; they are all connected with a spiral precapillary vein which runs under the tunnel of Corti. The pars pectinata of the basilar membrane is devoid of blood vessels.

The connective tissue of the spiral ligament contains numerous collagenous fibers and stellate connective tissue cells filled with pigment and other inclusions; it is also provided with numerous blood vessels.

Above the line of attachment of the basilar membrane to the spiral ligament, approximately at the same level as the vestibular lip of the limbus, the connective tissue forms a small ridge extending through the whole cochlea—the *prominentia spiralis*. It contains large capillary loops—the *vas prominens*. The groove between the ridge and the crest of the spiral ligament is the external spiral sulcus.

The thickness of the vestibular membrane is only 3 μ. It consists of an extremely thin connective tissue layer which in man has no blood vessels but sometimes contains pigment cells and very fine elastic networks. On the vestibular surface it is lined with the usual mesenchymal epithelium of the perilymphatic spaces.

The Epithelium of the Cochlear Duct. The ectodermal epithelium which lines the inner surface of the walls of the cochlear duct presents great differences in its various regions.

The inner surface of the vestibular membrane is covered with a simple squamous irregular epithelium often containing pigment. The surface of the limbus is lined with a mosaic of small, polygonal, cuticular plates belonging to epithelial cells whose bodies, containing the nucleus, are deeply sunk into the subjacent connective tissue The cells are arranged in radial rows along the furrows on the surface of the limbus, between the auditory teeth The internal spiral sulcus is lined with one layer of polygonal epithelial cells of medium thickness Outwardly they are followed by the inner border cells which belong to the organ of Corti.

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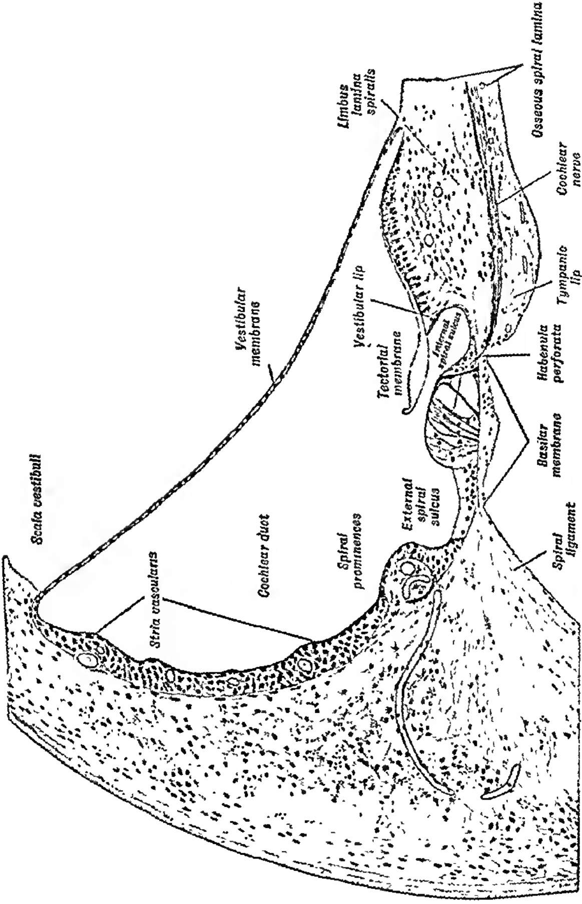

Fig. 556. Radial transection of the first coil of the human cochlea. The separation of the free end of the tectorial membrane from the organ of Corti is due to improper fixation. After Held.

upper surface, the tympanic lip, therefore, presents a regular row of holes; hence the name *habenula perforata*.

Farther outward, the tympanic lip continues into the *basilar membrane* (Fig. 557) which is tightly stretched between

this lip and the crest of the spiral ligament. The basilar membrane can be subdivided into an inner zone (zona arcuata) extending from the foramina nervosa to the base of the external pillars, and an outer zone (zona pectinata) between the external pillars and the crest of the spiral ligament.

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The epithelium covering the periosteum of the

outer, slightly concave wall of the cochlear duct and the part of the spiral ligament which faces the lumen of the cochlear duct is considerably thickened and is sometimes provided with irregular prominences. It extends downward to the external spiral sulcus and is called the *stria vascularis* (Fig. 556). It is of a low pseudostratified or stratified columnar type and shows a very irregular arrangement of cells. Many of them send out lobated processes into the connective tissue and some of them come in contact with the blood vessels. The mitochondria in their protoplasm are arranged in vertical rows in the basal part of the cells; the protoplasm also contains vacuoles, pigment granules and, in fetal life, glycogen.

In the subjacent connective tissue there are abundant capillaries which run for the most part in the spiral direction. They form loops which penetrate deeply into the epithelium and are sometimes accompanied by connective tissue cells.

It is believed that the stria vascularis secretes the endolymph of the cochlear canal. The organ of Corti has no blood vessels. Its elements must receive their nutritive materials and oxygen from the endolymph. As the organ of Corti does not display the phenomenon of peripheral fatigue, it is probable that the endolymph is constantly renewed.

In the external spiral sulcus the epithelium is cuboidal; its cells also have processes deeply penetrating into the connective tissue. According to some investigators this epithelium forms glandlike invaginations. Approaching the basilar membrane, the epithelium becomes more regular and continues upon the pars pectinata in the form of a layer of clear polygonal elements of varying height—the *cells of Claudius* (Fig. 557). In some places of the basal coil, small groups of dark, polyhedral cells are scattered between the basilar membrane and the cells of Claudius—the *cells of Boettcher.*

The Organ of Corti or the Papilla Basilaris. Outwardly from the tympanic lip of the limbus, the basilar membrane carries on its upper surface an epithelial ridge of considerable thickness and of extremely complex structure—the organ of Corti or the papilla basilaris. It extends spirally throughout the length of the cochlear duct. In a radial section it has the form of an irregularly trapezoid prominence bulging into the lumen of the cochlear duct (Fig. 557). Among its elements two types have to be distinguished: (1) The sustentacular or supporting cells which form a rigid, but flexible framework supporting the whole structure and (2) the hair cells—neuro-epithelial elements which act as receptors of the stimuli produced by the sound waves. All these elements are arranged in regular, longitudinal (spiral) rows.

Supporting Cells. There are different types of supporting cells, but certain characteristic features are common to all of them. They are slender, tall elements which contain rigid tonofibrils in their protoplasm and extend from the basilar membrane through the whole thickness of the epithelium to the free surface of the organ of Corti. Here their substance expands to form a cuticular plate. Whereas the thin rigid bodies are separated from one another by large intercellular spaces, the cuticles are intimately connected with one another by their edges in a regular mosaic and form a continuous, cuticular membrane covering the surface of the organ. Owing to the peculiar shape of the cuticular plates the membrane has many holes arranged in regular, alternating rows and is, therefore, called the *reticular membrane.* In the holes the round cuticular plates of the hair cells are fastened. The bodies of these elements are suspended in the intercellular spaces and do not reach the basilar membrane.

Among the supporting cells the following types have to be distinguished: (1) The inner and outer pillars or rods; (2) the inner and outer phalangeal cells; (3) the border cells; (4) the cells of Hensen.

The Pillars or Rods. The central structure of the organ of Corti is the *tunnel* (Fig. 557). It is a canal, extending through the whole length of the cochlea and having a triangular shape in a radial transection. One side of the triangle is formed by the basilar membrane; the other two sides, converging toward the surface of the epithelium, are formed by the inner and outer pillar cells. Toward the upper coil of the cochlea the upper angle of the tunnel gradually becomes larger.

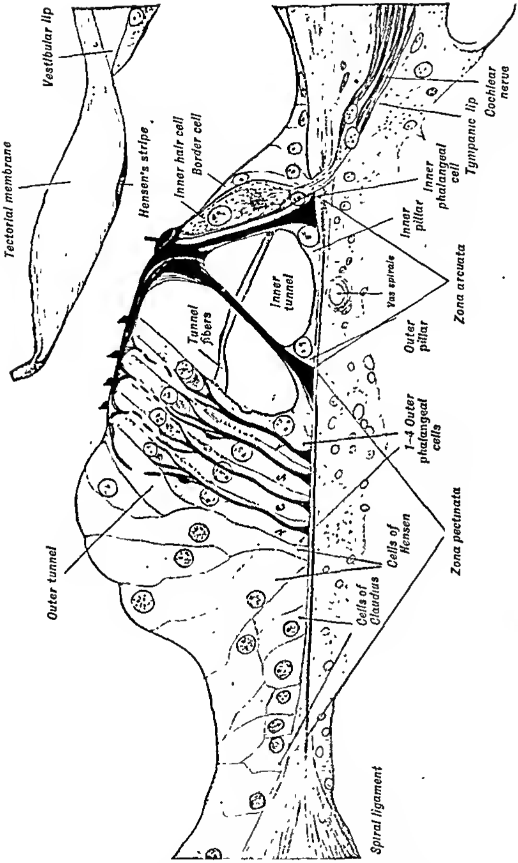

Fig. 557. Radial transection of the human organ of Corti, from the upper part of the first coil. The separation of the tectorial membrane is an artefact. After Held.

In the slanting side walls of the tunnel the thin bodies of the pillars are separated from one another by cleftlike spaces, as in the paling of a fence Through these openings the cavity of the tunnel communicates with the other intercellular cavities in the organ of Corti. The roof of the

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Among the supporting cells the following types have to be distinguished: (1) The inner and outer pillars or rods; (2) the inner and outer phalangeal cells; (3) the border cells; (4) the cells of Hensen.

The Pillars or Rods. The central structure of the organ of Corti is the *tunnel* (Fig. 557). It is a canal, extending through the whole length of the cochlea and having a triangular shape in a radial transection. One side of the triangle is formed by the basilar membrane; the other two sides, converging toward the surface of the epithelium, are formed by the inner and outer pillar cells. Toward the upper coil of the cochlea the upper angle of the tunnel gradually becomes

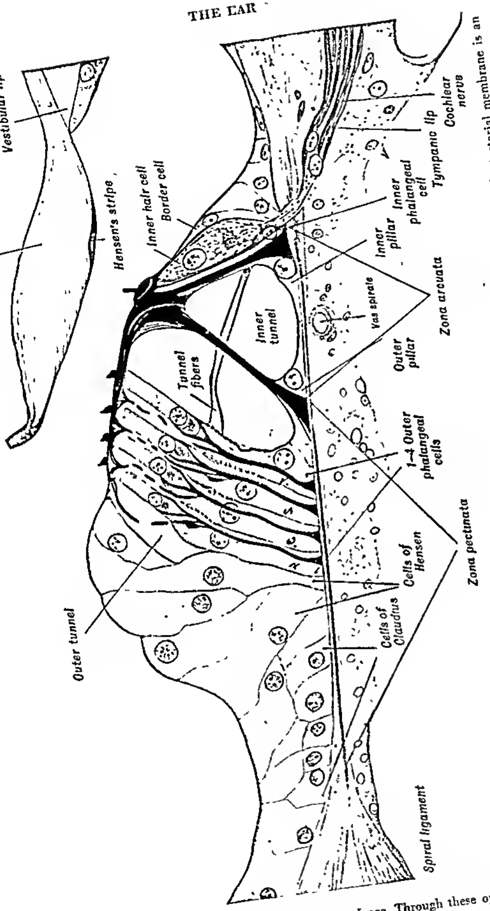


Fig 557. Radial transection of the human organ of Corti, from the upper part of the first coil. The separation of the tectorial membrane is an artefact After Held.

In the slanting side walls of the tunnel the thin bodies of the pillars are separated from one another by cleftlike spaces, as in the paling of a fence. Through these openings the cavity of the tunnel communicates with the other intercellular cavities in the organ of Corti. The roof of the

tunnel, where the inner and outer rods are intimately connected with one another, is continuous and forms a part of the reticular membrane.

The Inner Pillars. The inner pillars have a broad polygonal base which rests on the basilar membrane outside of the foramina nervosa; the cell body rises conically upward from this base. The cellular substance at the inner corner of the tunnel is undifferentiated protoplasm and contains a round nucleus. The rest of it is formed by a small, solid, conical condensation and by rigid, darkly staining tonofibrils which surround the cone and assemble above it to form a compact, cylindrical bundle. This bundle forms the *slender body of the pillar* (2 to 3 μ thick) and is ensheathed by a trace of protoplasm. On approaching the surface the pillar again becomes thicker and ends with the head, covered on its free surface by a cuticular plate. In the substance of the head the fibrils spread out and are attached to the inner and outer edge of the cuticle. The latter is a narrow, radially elongated rectangle. Its long side edges are connected with the cuticles of the neighboring inner pillars. The small inner margin is slightly concave and fits the outer periphery of the cuticle of an inner hair cell. The outer small edge overlies the cuticular plates of the outer pillars and contains a diplosome. The outer surface of the head beneath the cuticle is excavated.

The Outer Pillars. The outer pillars are longer than the inner ones and are slightly curved in the fashion of an S. Their base is attached to the basilar membrane at the junction of the zona arcuata with the pectinata; it is similar to the base of the inner pillars and also consists of a small accumulation of protoplasm with a nucleus which occupies the outer corner of the tunnel; it also continues into a long slender body with a bundle of tonofibrils. The head of the outer pillar has a convex inner surface which fits the excavation on the outer surface of the head of the inner pillar and is firmly attached to it. The *cuticular plate* projects outwardly from under the cuticle of the inner pillar and has a typical oar- or shovel-like shape; at its outer end it contains the diplosome. The oar-shaped cuticular plates of the outer pillars form the first (innermost) row of phalanges. The holes between their excavated edges contain the first row of the outer hair cells. In the same way as in the inner pillars, the tonofibrils reaching the head spread fanlike and are attached to the surface and to the edge of the phalange.

The *number of the inner pillars* (5600) is higher than that of the outer ones (3800). On the average three inner pillars are connected with two outer pillars.

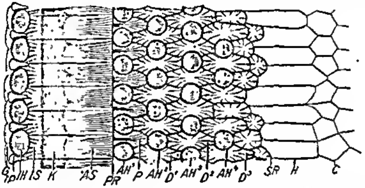


Fig. 558. View from above of the papilla spiralis with the supporting fiber system, hair cells, centrioles and the membrana reticularis; semischematic (partly after Retzius and Kolmer). *AH¹–AH⁴*, outer hair cells; *AS*, head plate of the inner pillar; *C*, cells of Claudius; *D¹–D³*, phalanges of Deiters' cells; *G*, border cells; *H*, cells of Hensen; *IH*, inner hair cells; *IP*, inner phalanges; *IS*, inner pillar; *P*, phalangeal continuation of the outer pillar; *PR*, cuticular margin of the head plates; *SR*, limiting membrane From Schaffer.

The Phalangeal Cells. These elements have a very complex form and structure which can be understood only by comparison of the pictures seen in radial and tangential sections with those in teased preparations, where the cells are more or less successfully isolated. Inner and outer phalangeal cells have to be distinguished.

The Inner Phalangeal Cells. The inner cells are slender elements, arranged in a row attached to the inner surface of the inner pillars. Their small angular bases occupy the narrow space between the bases of the inner pillars and the foramina nervosa on the basilar membrane. The cell body contains a nucleus in its lower part and a slender bundle of tonofibrils. It mounts to the surface and ends here with a small cuticular plate containing the diplosome. The plate is elongated in the radial direction and both of its

long edges are excavated; its form resembles that of the small bones of the fingers, the *phalanges.* This explains the origin of the name given to these cells. The outer ends of the phalanges of the inner phalangeal cells are connected with the pointed inner ends of the cuticles of the inner pillars. Their excavated side edges, together with the excavations of the plates of the inner pillars on the outer and the plates of the border cells on the inner side, circumscribe the openings in which the cuticular plates of the inner hair cells are fastened.

The Outer Phalangeal Cells (Deiters). The outer phalangeal cells, the cells of Deiters, form three regularly alternating rows outside of the row of the outer pillars. Their arrangement is adapted to that of the outer hair cells which also form three rows. In the second coil, where there are four lines of hair cells, a fourth line of outer phalangeal cells appears. In the third coil a fifth, usually irregular and interrupted row of hair cells and of slightly atypical phalangeal cells is added.

The hexagonal bases of the cells of Deiters pave the basilar membrane outside of the row of the outer pillars. The cell body is prism-shaped; its protoplasm contains a nucleus and is very difficult to preserve. Through its axis runs a rigid bundle of fibers. They begin in the middle of the base with a fibrillar, conical pedestal. The body suddenly tapers down toward the surface and continues as a thin stalk formed by the fibrillar bundle. It mounts to the surface and here expands into a radially directed phalange with symmetrical, semicircular excavations on its long side edges; these fit the cuticles of the hair cells of the corresponding row. When the fibrils of the supporting fiber reach the surface they expand beneath the phalange and end at its smaller outer and inner margins. Near its outer margin the phalange contains a diplosome.

The phalanges of one row interdigitate with the phalanges of the new row. Since the outer pillars form the first (innermost) row of external phalanges, the second row of phalanges belongs to the first row of the cells of Deiters, the third to the second, the fourth to the third row of cells.

The phalanges of the different orders, tightly fastened together along their edges by an extensive system of terminal bars, constitute the above-mentioned reticular membrane with its regular mosaic pattern and rows of alternating round openings containing the cuticles of the hair cells. The outermost phalanges (belonging according to the level of the cochlea to the third, fourth or fifth row of Deiters' cells) have a simple, sometimes irregular polygonal shape; they form the outer edge of the reticular membrane—its *terminal frame.*

At the place where the cell body of the outer phalangeal cell begins to taper into the thin stalk, the inner surface of the protoplasm forms a prominence; into it extends a separate bundle of fibers which branches off from the main bundle. The surface of this bulging is excavated and lodges the lower, rounded end of a hair cell.

In a radial section of the cochlea the cells of Deiters are never seen in their full length. The prismatic cell body is cut off at the level where it begins to taper. Above the stump containing the nucleus, portions of several overlapping processes of phalangeal cells and, on the surface, a phalange can be seen. These parts belong to other phalangeal cells of the same row which are located farther down toward the base of the cochlea. A full view of a cell of Deiters can be had only in a tangential section which runs parallel to the axis of the modiolus. Here it is seen that the slender phalangeal process of each cell is bent toward the apex of the cochlea, *i.e.,* upward in the spiral direction, and that it passes three hair cells before reaching the surface and ending with its phalange. In the outermost row of these cells the inclination is much less marked and the process of each cell crosses only one hair cell.

Whereas the basal parts of the phalangeal cells are closely adjacent to one another there are large intercellular intra-epithelial spaces—the *spaces of Nuel* between their slender processes. An especially conspicuous space is found between the outer pillars and the first row of phalangeal cells. The space between the two outer rows of phalangeal cells is sometimes called the *outer tunnel.*

Through the clefts between the outer pillars the spaces of Nuel communicate with the interior of the tunnel. All these spaces are believed to be filled with a peculiar, gelatinous, intercellular substance.

The Border Cells. These slender elements, with a nucleus in the lower or upper part of the cell body, stand in a row inside of the foramina nervosa and the inner hair cells (Fig. 557). Their narrow cuticles, provide with a diplosome, are arranged in a row which closes from within the openings which contain the inner hair cells. They form the inner margin of the reticular membrane.

The protoplasm of the basal part of these cells sends out processes which accompany and support the nerve fibers in the organ of Corti. Toward the inner spiral sulcus the border cells are followed by cells which rapidly diminish in size and pass into squamous cells.

The Cells of Hensen. The cells of Deiters

are succeeded in the outward direction by the very tall cells of Hensen which have a small base and an enlarged upper part (Fig. 557). The latter bulges on the surface at the outer edge of the organ and contains the nucleus. It is often filled with fat droplets and pigment granules. As these cells are slightly inclined in the spiral direction toward the apex of the cochlea they usually overlap in radial sections. The cells of Hensen are arranged in several rows: in the outward direction they rapidly decrease in height and pass into the *cells of Claudius.*

The Hair Cells. The free surface of the hair cells, fastened in the holes of the reticular membrane, is provided with short, rigid, bristle-like outgrowths. There are two kinds of these elements.

The inner hair cells are arranged in one row between the inner phalangeal and the border cells on the one hand and the inner pillars on the other (Fig. 558). The outer hair cells form three rows and are suspended between the outer pillars and the cells of Deiters. In the second coil a fourth, in the upper coil a fifth row of outer cells is added.

All the hair cells have a short cylindrical shape with a rounded lower end which contains the nucleus and with a cuticular plate on the free surface. The cuticle of the inner hair cells is oval and has its long diameter in the spiral direction. The cuticle of the outer cells is usually slightly elongated in the radial direction.

Immediately under the cuticle the protoplasm contains a modified Golgi net—the *body of Hensen.* Under the nucleus, at the lower end of the cell, a condensed protoplasmic mass with pigment granules, the *body of Retzius,* can be seen.

The cuticle of the inner hair cells is surmounted by 41 to 64 hairs arranged in two or more straight, parallel rows in the longitudinal (spiral) direction. At the outer edge of the cuticle lies the diplosome with a minute flagellum. The hairs of the outer cells (83 to 100 hairs to a cell) form several parallel, horseshoe-shaped lines, with the convexity directed outward. The length of these hairs decreases from outside inward. The outer edge of the cuticle contains the diplosome.

The Tectorial Membrane. The surface of the organ of Corti is covered by a peculiar ribbon-like structure of jelly-like consistency—the tectorial membrane. It is very difficult to preserve it without causing artificial distortions. In fixed slides its outer free edge is usually more or less curled up or the whole membrane is detached from the epithelium (Fig. 557).

The width of the membrane increases toward the apex of the cochlea. In a radial section it has a planoconvex form. It begins in the inner angle of the cochlear canal as a thin, cuticular layer firmly attached to the surface of the epithelium of the limbus. Farther outward, where the membrane freely overhangs the inner spiral sulcus and then touches the organ of Corti, its lower surface remains even, while the upper one bulges considerably upward. Here the thickness of the membrane reaches 25 μ. Passing over the organ of Corti it again becomes thinner and after having reached the cells of Hensen it ends with a perforated, lacelike, irregularly fringed edge.

In the substance of the tectorial membrane, a homogeneous jelly-like ground substance and numerous fine fibrils have to be distinguished. The fibers are most conspicuous at the upper surface where they anastomose and form networks. In the deeper layers they are more or less regularly parallel; their general direction is radial, but with a marked deviation from the radial plane toward the apex of the cochlea. Along the lower surface of the membrane, opposite the row of inner hair cells, a darker stripe, the *stripe of Hensen,* extends in the spiral direction.

In the living condition the lower surface of the tectorial membrane rests lightly upon the ends of the hairs which protrude from the hair cells. In well fixed

preparations the lower surface of the tectorial membrane is attached to the ends of the hairs projecting from the hair cells.

The Nerve Endings in the Organ of Corti. Except for the possible existence of vasomotor nerves in the labyrinth, the end branches of the cochlear nerve are the only nerve fibers which can be found in the cochlea. The existence of centrifugal fibers of unknown origin ending in the organ of Corti is doubtful; these are, however, present in the modiolus and osseous spiral lamina, and are probably sympathetic.

The nerve cells of the spiral ganglion are of the bipolar type; their central processes, continuing into the acoustic nerve, can be considered as axons. The peripheral processes which run through the canals of the osseous lamina and through the foramina nervosa toward the hair cells are dendrites. Both processes have a myelin sheath with lemmoblasts. A thin layer of myelin is also found on the surface of the cell body. The peripheral fibers keep the myelin until they reach the foramina nervosa. In passing through them they lose the myelin and enter the organ of Corti, where they finally reach the hair cells.

There are essentially two kinds of nerve fibers in the organ of Corti, each terminating in a special way. The first, which are thinner and more numerous, reach from their respective segments of the spiral ganglion in radiating, parallel bundles to the nearest segments of the basilar papilla. Here each fiber at once divides into a number of small branchlets terminating with buttons attached to the surface of the hair cells. Because of their straight course these may be called *direct acoustic nerve fibers* (orthoneurons of v. Ebner). In these each nerve fiber is related to a compact group of hair cells whence it receives stimulation.

The other and usually thicker nerve fibers, although fewer in numbers than the first, are conspicuous because of their peculiar course. They, too, are at first arranged radially. After reaching the territory of the basilar papilla they sharply turn in a longitudinal direction and lie in parallel bundles beneath and between the several rows of hair cells and the various supporting structures. Here they form several so-called "plexuses," one underneath the inner hair cells, one in the inner tunnel, and several between the outer phalangeal cells of Deiters. Because of their course these fibers are called *spiral fibers* (spironeurons of v. Ebner). They, too, terminate with tree-shaped branchings, larger than in the direct fibers, each spiral fiber being related to a compact group of hair cells. The group of cells is always at a certain distance along the basilar papilla from the point where the fiber changes from the radial into the spiral course. The spiral fibers, while in the basilar papilla, as a rule turn toward the basal coil of the cochlea.

The presence of the two varieties of the acoustic nerve fibers in the organ of Corti announced by earlier investigators (Retzius, Ramón y Cajal) but doubted by others (Kolmer, Lorente de Nó) is now sufficiently well ascertained (Polyak, Tello). Less certain is the interpretation of such an arrangement in terms of function. Although the relations between the peripheral receptors, the hair cells, and the acoustic neurons are not as individualized as the monosymnaptic relationships in the foveal cones (b-h relationship, see p. 624), they are nevertheless sufficiently restricted as to permit the reception of localized stimuli impinging upon small segments of the cochlea. This applies both to the system of the direct and of spiral fibers. The significance of the spiral fibers is unknown. Possibly, in collaboration with the groups of direct fibers, the spiral fibers in some way serve in the process of the reception of complex sounds, whereas the system of direct fibers alone is instru-

mental in the perception of simple tones.

In any event, the cochlea, like the retina, must be considered to be an organ with a receptor surface whose different points possess different functional value. This view agrees with the facts concerning the cerebral projection of the cochlea, recently revealed. These show that the basilar membrane is not represented in the brain in a summary way but topically, or point for point (Polyak, Lewi and Kobrak, Walker).

Nerves of the Labyrinth. The eighth cranial nerve supplies the sensory areas of the labyrinth. It consists of two parts of quite different functional nature and central connections—the *vestibular* and the *cochlear* nerves. The first divides into a superior and an inferior branch. The fibers of all these nerves are the central processes (axons) of bipolar ganglionic cells which form two ganglia. Of these, the spiral or cochlear ganglion is incorporated in the cochlear nerve and located in the modiolus of the cochlea, while the vestibular ganglion belongs to the vestibular nerve and is located in the inner auditory meatus of the temporal bone. In correspondence with the subdivision of the vestibular nerve into a superior and an inferior branch the vestibular ganglion can be also more or less distinctly separated into a superior and an inferior part. The peripheral branches (dendrites) of the bipolar ganglionic cells run to the sensory areas. The superior vestibular ganglion sends out four branches which supply: (1) the horizontal or lateral ampulla; (2) the frontal or superior ampulla; (3) the utricular macula; and (4) a small part of the saccular macula. From the inferior vestibular ganglion three branches arise. The first, the largest, supplies the larger part of the saccula macula; the second, the sagittal or posterior ampulla; the third, the smallest, joins the fibers of the cochlear nerve; the latter connection needs further study (Fig. 557).

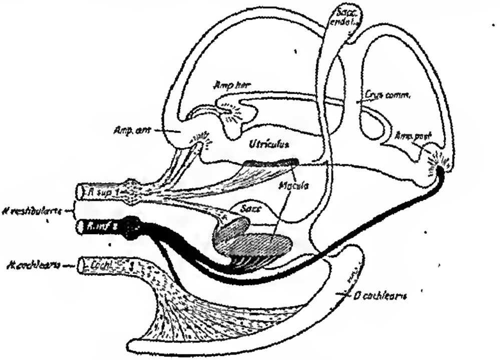


Fig. 559. Diagram of the distribution of the nerves in the membranous labyrinth of the rabbit. After de Burlet from Kolmer.

The vestibular nerve terminates centrally in the reflex centers of the medulla oblongata and the cerebellum. Its cortical connections are unknown. The cochlear nerve also has reflex connections in the medulla oblongata and the midbrain; but most of its fibers run in the lateral lemniscus to the medial geniculate body of the thalamus and thence to the sylvian fossa of the brain center for hearing.

Blood Vessels of the Labyrinth. The labyrinthine artery is a branch of the inferior cerebellar artery. It enters the internal meatus and divides into two branches, the vestibular artery and the common cochlear artery. The latter divides into the vestibulocochlear artery (Fig 560) and the cochlear artery proper.

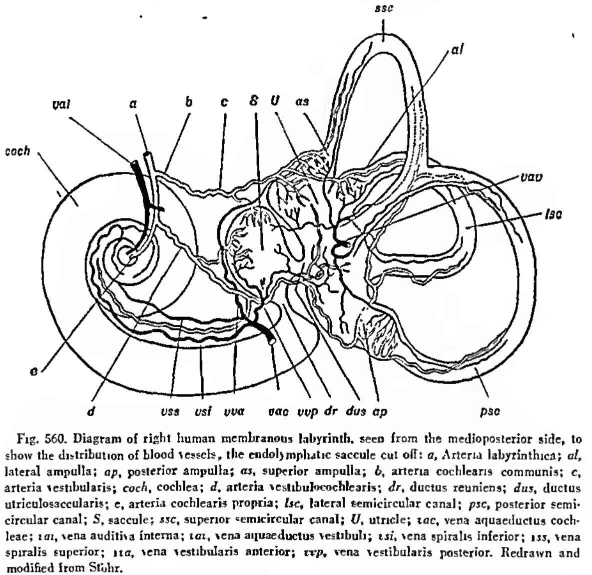
The vestibular artery supplies the upper and lateral parts of the utricle and saccule and parts of the superior and lateral semicircular canals. It forms dense networks of capillaries in the

region of the maculae; in the narrow connective tissue layer of the thin walls of these structures the capillary networks are very loose.

The vestibulocochlear artery supplies the lower and medial parts of the utricle and saccule, the crus commune, and the posterior semicircular canal with its vestibular branch. Its cochlear branch supplies the lowest part of the first cochlear coil.

The cochlear artery proper penetrates into the tioned. The vascular stria and the spiral ligament receive their blood through branches of the spiral arterial tract which run in the roof of the scala vestibuli. They do not form connections with the vessels of the basilar membrane. The lower wall of the scala tympani receives its own small arteries from the same source.

The course of the veins of the labyrinth is quite different from that of the arteries. There are three draining, venous systems.



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cavities of the modiolus; here its branches form many irregular zigzags and coils and run spirally to the apex; this is the so-called "spiral tract." From the latter, side branches are sent out to the spiral ganglion and, through the periosteum of the scala vestibuli and the spiral osseous lamina, to the inner parts of the basilar membrane. Here the capillaries are arranged in the form of arcades in the tympanic covering layer under the tunnel and the limbus; from them arises the vas spirale which has just been men-

In the cochlea the veins originate in the region of the prominentia spiralis and run downward and inward through the periosteum of the scala tympani to the spiral vein which is found under the spiral ganglion; the spiral vein also receives branches from the osseous spiral lamina and from the spiral ganglion. Two such spiral veins can be distinguished—the upper and the lower; they belong to the corresponding coils of the cochlea. Above the spiral vein is the small vein of the spiral lamina which receives a part of the blood

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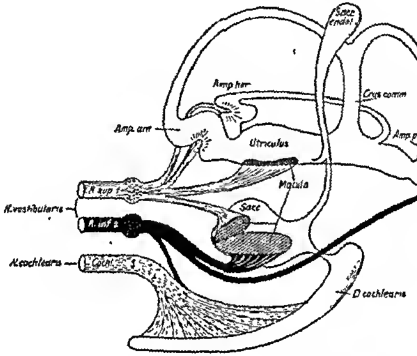

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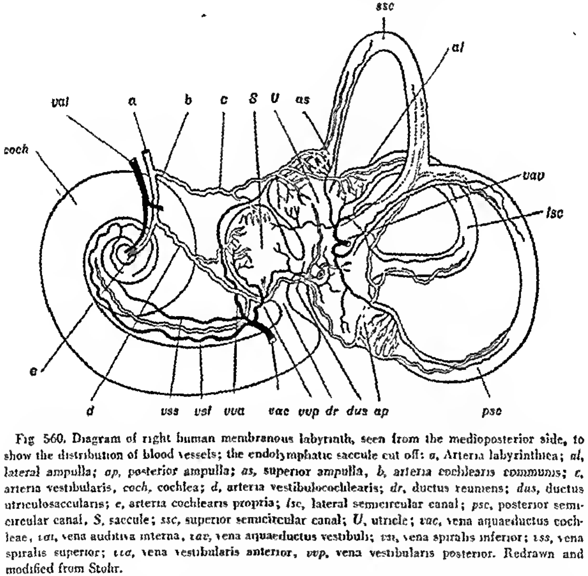

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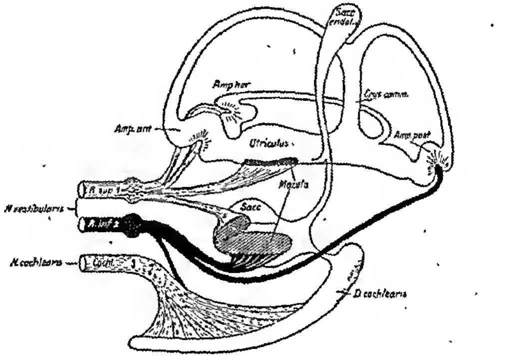

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The vestibular artery supplies the upper and lateral parts of the utricle and saccule and parts of the superior and lateral semicircular canals. It forms dense networks of capillaries in the

region of the maculae; in the narrow connective tissue layer of the thin walls of these structures the capillary networks are very loose.

The vestibulocochlear artery supplies the lower and medial parts of the utricle and saccule, the crus commune, and the posterior semicircular canal with its vestibular branch. Its cochlear branch supplies the lowest part of the first cochlear coil.

The cochlear artery proper penetrates into the cavities of the modiolus; here its branches form many irregular zigzags and coils and run spirally to the apex, this is the so-called "spiral tract" From the latter, side branches are sent out to the spiral ganglion and, through the periosteum of the scala vestibuli and the spiral osseous lamina, to the inner parts of the basilar membrane Here the capillaries are arranged in the form of arcades in the tympanic covering layer under the tunnel and the limbus, from them arises the vas spirale which has just been mentioned. The vascular stria and the spiral ligament receive their blood through branches of the spiral arterial tract which run in the roof of the scala vestibuli. They do not form connections with the vessels of the basilar membrane. The lower wall of the scala tympani receives its own small arteries from the same source.

The course of the veins of the labyrinth is quite different from that of the arteries. There are three draining, venous systems.

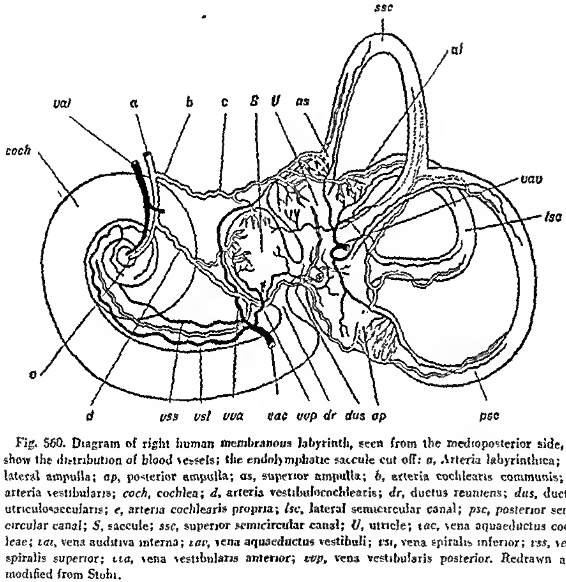

Fig. 560. Diagram of right human membranous labyrinth, seen from the medioposterior side, to show the distribution of blood vessels; the endolymphatic saccule cut off: *a*, Arteria labyrinthica; *al*, lateral ampulla; *ap*, posterior ampulla; *as*, superior ampulla; *b*, arteria cochlearis communis; *c*, arteria vestibularis; *coch*, cochlea; *d*, arteria vestibulocochlearis; *dr*, ductus reuniens; *dus*, ductus utriculosaccularis; *e*, arteria cochlearis propria; *lsc*, lateral semicircular canal; *psc*, posterior semicircular canal; *S*, saccule; *ssc*, superior semicircular canal; *U*, utricle; *vac*, vena aquaeductus cochleae; *vai*, vena auditiva interna; *vav*, vena aquaeductus vestibuli; *vsi*, vena spiralis inferior; *vss*, vena spiralis superior; *vva*, vena vestibularis anterior; *vvp*, vena vestibularis posterior. Redrawn and modified from Stohr.

In the cochlea the veins originate in the region of the prominentia spiralis and run downward and inward through the periosteum of the scala tympani to the spiral vein which is found under the spiral ganglion; the spiral vein also receives branches from the osseous spiral lamina and from the spiral ganglion. Two such spiral veins can be distinguished—the upper and the lower; they belong to the corresponding coils of the cochlea. Above the spiral vein is the small vein of the spiral lamina which receives a part of the blood

mental in the perception of simple tones.

In any *event*, the cochlea, like the retina, must be considered to be an organ with a receptor surface whose different points possess *different functional* value. This view agrees with the facts concerning the cerebral projection of the cochlea, recently revealed. These show that the basilar membrane is not represented in the brain in a summary way but topically, or point for point (Polyak, Lewi and Kobrak, Walker).

Nerves of the Labyrinth. The eighth cranial nerve supplies the sensory areas of the labyrinth. It consists of two parts of quite different functional nature and central connections—the *vestibular* and the *cochlear* nerves. The first divides into a superior and an inferior branch. The fibers of all these nerves are the central processes (axons) of bipolar ganglionic cells which form *two ganglia*. Of *these*, the spiral or cochlear ganglion *is incorporated in the cochlear nerve* and located in the *modiolus* of the cochlea, while the vestibular ganglion belongs to the vestibular nerve and is located in the inner auditory meatus of the temporal bone. In correspondence with the subdivision of the vestibular nerve into a superior and an inferior branch the vestibular ganglion can be also more or less distinctly separated into a superior and an inferior part. The peripheral branches (dendrites) of the bipolar ganglionic cells run to the sensory areas. The superior vestibular ganglion sends out four branches which supply: (1) *the horizontal or* lateral ampulla; (2) the *frontal or superior* ampulla; (3) the utricular macula; and (4) a small part of the saccular macula. From the inferior vestibular ganglion three branches arise. The first, the largest, supplies the larger part of the saccula macula; the second, the sagittal or posterior ampulla; the third, the smallest, joins the fibers of the cochlear nerve; the latter connection needs further study (Fig. 557).

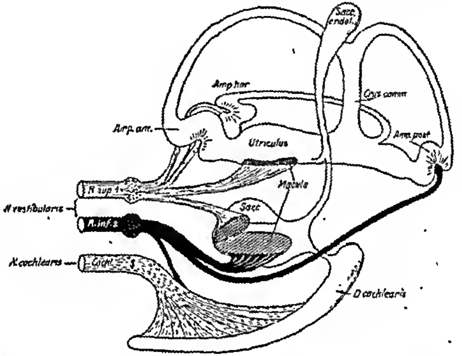

Fig. 559. *Diagram of the distribution of the nerves in the membranous labyrinth of the rabbit.* After de Burlet from Kolmer.

The vestibular nerve terminates centrally in the reflex centers of the medulla oblongata and the cerebellum. Its cortical connections are unknown. The cochlear nerve also has reflex connections in the medulla oblongata and the midbrain; but most of its fibers run in the lateral lemniscus to the medial geniculate body of the thalamus and thence to the sylvian fossa of the brain center for hearing.

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The vestibular artery supplies *the upper* and lateral parts of the utricle and saccule and *parts* of the superior and lateral semicircular *canals.* It forms dense networks of capillaries in the

arteries are so prominent that they make the impression of convoluted tufts (glomeruli).

True lymphatics are absent in the labyrinth. Instead, the excess of the tissue liquid is drained into the perilymphatic spaces which are connected through the cochlear aqueduct with the subarachnoid spaces. A certain amount of drainage may be effected through the perivascular and perineural connective tissue sheaths.

Histophysiologic Remarks. The tympanic membrane, besides protecting the middle ear, receives the sound waves and transmits them to the auditory ossicles.

Function of the Cochlea. The vibrations of the tympanic membrane are transmitted through the chain of the auditory ossicles to the fenestra ovalis and hence to the perilymph filling the scala tympani. The organ of Corti is the receptor for sound stimuli. It is generally believed that the analysis of the sound waves is accomplished in the organ of Corti and that this depends upon the basilar membrane, a mechanism for *sympathetic vibration* or *resonance*.

Function of the Vestibulum The nervous impulses elicited by the stimulation of the maculae and the cristae play an important *rôle* in the regulation and coordination of the movements of equilibrium and locomotion. The main brain center through which these impulses exert their influence upon coordinated muscular contractions, upon muscular tonus, and upon the eyes, is the brain stem and the cerebellum.

Histogenetic Remarks. *The External and Middle Ears.* The tympanic cavity and the auditory tube are derivatives of the first branchial pouch. The external auditory meatus develops through an invagination of the integument directed toward the tympanic cavity. The tissue layer remaining between this invagination and the tympanic cavity is transformed into the tympanic membrane.

The Otic Vesicle. The primordium of the labyrinth develops as a shallow groove of thickened ectodermal epithelium, dorsally to the first branchial groove, on both sides of the brain, between the myelencephalon and the metencephalon (human embryo of 8 somites). The groove is invaginated into the subjacent mesenchyme and is transformed into the *otic vesicle*. In a human embryo of 2.8 mm. it separates through constriction from the ectoderm and is surrounded by mesenchyme. The cavity of the vesicle is lined by tall pseudostratified epithelium which secretes the endolymph filling the vesicle.

From its earliest stages the otic vesicle comes into intimate contact with the large acoustic ganglion which originates from the neighboring ganglionic crest The ganglion adheres to the epithelium at the anterior side and later at the medial side of the vesicle. It later divides into the vestibular and cochlear ganglia. Unequal proliferation in different places of the epithelial wall of the otic vesicle leads to the formation of folds, evaginations and constrictions, and transforms it into an extremely complex system of sac- and tubelike cavities.

Soon after the isolation from the ectoderm the dorsal periphery of the vesicle sends out an evagination which is the primordium of the endolymphatic duct. Then a larger dorsal part of the vesicle becomes distinctly separated from a smaller ventral part. The first—the vestibular part—gives rise to the semicircular canals and the utricles. The second, the cochlear part, forms the saccule and the cochlea.

On the wall of the vestibular part three flattened, foldlike evaginations appear; and develop into the three semicircular canals each with an ampulla. What remains of the vestibular part is now the utricle.

The cochlear part sends out a slightly curved outpocketing—the primordium of the cochlea. It gradually gains in length, coils up as it grows, and becomes separated from the rest of the cochlear part, the saccule, by a deep constriction. In a human embryo of 22 mm. the form of the labyrinth corresponds to that of the adult.

Maculae and Cristae. The maculae and cristae develop earlier than the organ of Corti. The epithelium of the wall of the otic vesicle develops, on its medial side, where the acoustic ganglion is located, a thickened area, the *common macula* (*macula communis*) which later divides into an upper and a lower epithelial pad The first gives rise to the macula of the utricle and to the upper and lateral cristae A small part of the second thickening forms the crista of the posterior ampulla; the rest of the second pad divides into the macula sacculi and the primordium of the organ of Corti which gradually extends into the growing cochlea.

In the maculae and cristae of a human embryo of 15 mm. two kinds of epithelial cells can already be distinguished—the plumper and darker neuroepithelial and the more slender supporting cells. In an embryo of 18.5 mm. the hair cells on their free surface have developed a small tuft of hairs. They as well as the supporting cells have on their surface a flagellum connected with a diplosome.

The cupula and the otolithic membrane arise in the center of the respective epithelial surface, when the two cell types just begin to differentiate, while at their edge the maculae or cristae are still growing. The membrane and the cupula appear first as a thin homogeneous layer secreted on the epithelial surface. Then, new layers of a jelly-like substance are added by the supporting

from the spiral lamina and from the spiral ganglion and is connected by anastomoses with the spiral vein. These veins of the cochlea form a plexus in the modiolus which empties the blood partly into the vena auditiva interna, which

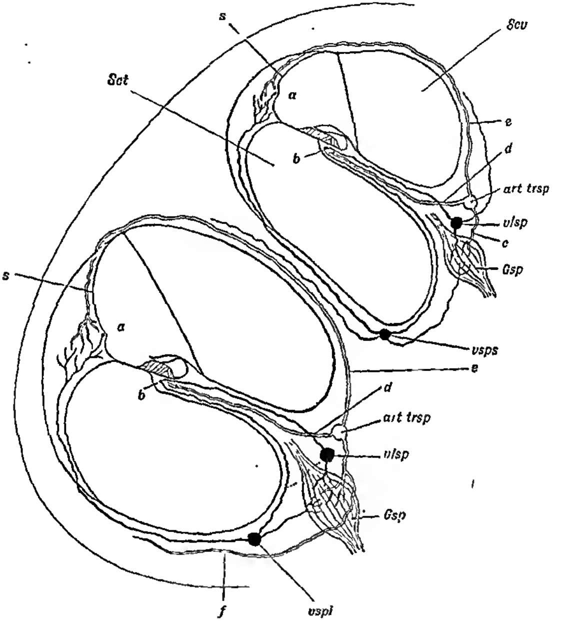

Fig. 561. Diagrammatic radial transection of the two lower coils of the cochlea showing the distribution of the blood vessels, arteries with double contours, veins black. *art trsp*, Artery of spiral tract; *a*, prominentia spiralis; *b*, vas spirale; *c*, artery supplying the spiral ganglion; *d*, artery supplying the spiral lamina; *e*, artery circling the upper wall of the scala; *f*, artery supplying partition between two coils of the cochlea; *Gsp*, ganglion spirale; *s*, stria vascularis; *Sct*, scala tympani; *Scv*, scala vestibuli; *vlsp*, vein of the spiral lamina; *vspi*, inferior spiral vein; *vsps*, superior spiral vein. Redrawn and modified from Stohr.

leaves the labyrinth with the labyrinthine artery, and partly into the vein of the cochlear aqueduct, which drains into the jugular vein

The veins of the vestibular apparatus and the semicircular canals empty into the veins of the vestibular and of the cochlear aqueducts.

This arrangement of the vessels in the internal ear seems to insure the best possible protection of the receptors of the sound stimuli from the concussions caused by the pulsating waves. The arteries are arranged, for the most part, in the wall of the scala vestibuli while the wall of the scala tympani contains the veins. The basilar membrane in its outer part, the zona pectinata, as well as the vestibular membrane, is completely devoid of blood vessels. The coiled course of the spiral arteries in the modiolus probably also contributes to the damping of the pulsatory concussions. In certain mammals the coils of these

the perilymphatic spaces, develop around the membranous labyrinth. They are especially large around the saccule and utricle, where the process starts. The perilymphatic spaces are traversed by strands of connective tissue which are the remnants of the loose mesenchyme. These trabeculae connect the cartilaginous capsule with the wall of the membranous labyrinth. Where the epithelium forms the sensory areas the mesenchyme remains dense. The mesenchymal cells which remain on the surface of the trabeculae and of the labyrinthine wall and perichondrium are flattened and are transformed into mesenchymal epithelium.

In tadpoles of the anura, if the otic vesicle is transplanted into another place of the body, it becomes surrounded by cartilage arising from the local mesenchyme.

The cochlea receives its perilymphatic spaces, the two scalae, through an extension of the perilymphatic cisterna along its upper and lower side. In embryos of 43 mm. the scala tympani appears in the region of the cochlear fenestra, in the stage of 50 mm. the scala vestibuli. They gradually grow out and coil together with the cochlear duct, always remaining attached to its upper and lower wall and compressing it between themselves, so that it acquires in transection a triangular shape. At the outer periphery of the cochlear duct, as well as at its inner edge, the mesenchyme is not loosened up. Here the wall of the duct remains connected with the cartilaginous capsule. Later ossification occurs which gives rise to the modiolus in the axis of the cochlear duct and to the outer wall of the osseous cochlea at its periphery.

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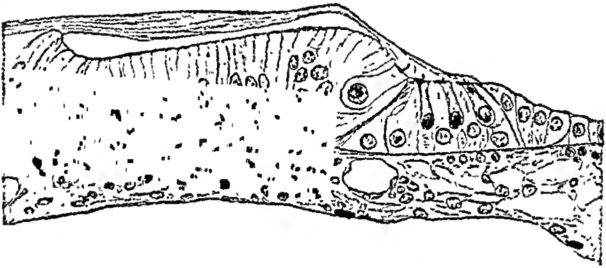
cells, whereas above the hair cells free spaces remain into which the growing hairs expand. The otoconia first arise as tiny, granular precipitates. They are believed to be secreted by the epithelium in the form of soluble calcium salts and to diffuse into the gelatinous layer; when they reach the surface of the latter they are precipitated in the form of crystals.

Histogenesis of the Organ of Corti. The differentiation of the organ of Corti proceeds gradually from the basal coil of the growing cochlea to its apex. The epithelium extends along the basal wall of the canal in the form of a long ridge which soon divides longitudinally into an inner larger and an outer smaller ridge. The fate of the tall cells of the inner ridge is different in its inner and outer parts. In the former, connective tissue penetrates into the epithelium and separates it into radial rows of flask-shaped cells embedded in connective tissue, while their cuticles form a mosaic pavement on the surface. This region develops into the limbus spiralis with its abrupt sharp edge and with its auditory teeth. In the outer part of the inner ridge the tall, slender epithelial cells in later stages gradually undergo a peculiar autolytic involution and disappear; only a thin layer of squamous cells remains—the epithelium which lines the internal spiral sulcus.

The *outer*, smaller ridge, which is the primordium of the organ of Corti, at first consists of uniform cells. Then among the latter dark, flask-shaped cells appear—the inner and outer hair cells. The remaining elements elaborate tonofibrils and differentiate into the various supporting cells. The spaces of Nuel as well as the lumen of the tunnel are the result of a partial dissolution of the protoplasm of the supporting cells in their middle height. On the surface their cuticles are closely connected into a continuous membrane.

The surface of both epithelial ridges is covered from the very beginning with a thin layer of a substance secreted by the epithelium and showing a fibrillar structure in a jelly-like ground substance. It is the future tectorial membrane. Its outer edge extends toward the outer surface of the outer ridge, where its fibers for a while seem to be firmly connected with the phalanges and the cells of Hensen. Inwardly it extends over the surface of the limbus and firmly adheres to the cuticle of the epithelial cells. When the cells of the inner ridge undergo autolysis and collapse, the tectorial membrane remains on the surface, spanning the gradually increasing excavation of the spiral sulcus.



... fetus of six months. The tectorial mem- ... with the surface of the ... ce. 311 ×. After Kolmer.

The connections between the sensory areas of the labyrinth and the nerve fibers of the vestibular and cochlear ganglia are established in very early stages. The pointed, free ends of the fibers, presumably guided by chemotactic influences, find their way into the thickened epithelial patches and form the endings described above.

Histogenesis of the Perilymphatic Space. While the otic vesicle grows and differentiates, the mesenchyme surrounding the epithelium of the growing labyrinth develops into a layer of cartilage, which remains separated from the epithelium by a layer of mesenchyme. This is later condensed to a fibrous layer and with the epithelium forms the wall of the membranous labyrinth. Between the wall and the cartilaginous capsule, the mesenchyme is loosened up and its meshes increase to such an extent that cavities filled with liquid

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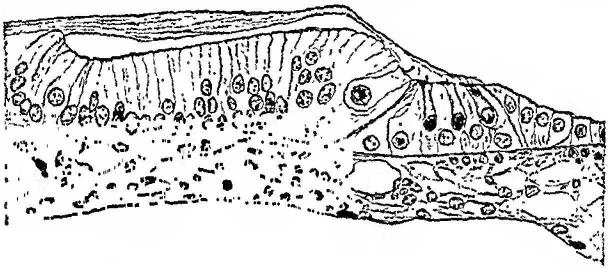

Fig. 562. Histogenesis of the organ of Corti in a human fetus of six months. Th
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